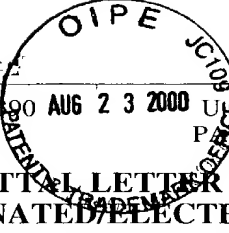


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INTERNATIONAL APPLICATION NO. PCT/US99/03790 ✓	INTERNATIONAL FILING DATE 22 February 1999 ✓	PRIORITY DATE CLAIMED 25 February 1998 ✓
TITLE OF INVENTION BEST'S MACULAR DYSTROPHY GENE ✓		
APPLICANT(S) FOR DO/EO/US Konstantin Petrukhin, C. Thomas Caskey and Michael Matzker ✓		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed [35 U.S.C. 371(c)(2)]. <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> A translation of the International Application into English [35 U.S.C. 371(c)(2)]. 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]. <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)]. 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)]. 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)]. <p>Items 11 to 16 below concern other document(s) or information included:</p> <ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input type="checkbox"/> Other items or information: 		

EXPRESS MAIL CERTIFICATE

DATE OF DEPOSIT August 23, 2000

EXPRESS MAIL NO. EK077635154115

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS
BEING DEPOSITED WITH THE UNITED STATES POSTAL
SERVICE AS EXPRESS MAIL "POST OFFICE TO
ADDRESSEE" BEFORE 5 P.M. ON THE ABOVE DATE IN
AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER
FOR PATENTS, WASHINGTON, D.C. 20231.

MAILED BY J. S. Crowley

DATE 8/23/2000

U.S. APPLICATION NO (If known, see 37 CFR 1.5) 09/622964	INTERNATIONAL APPLICATION NO. PCT/US99/03790	ATTORNEY'S DOCKET NUMBER 20177YP
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17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]: Neither international preliminary examination fee (37 CFR 1.482) nor international search fee [37 CFR 1.445(a)(2)] paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$970.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$840.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee [37 CFR 1.445(a)(2)] paid to USPTO \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$670.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) .. \$96.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>	CALCULATIONS	PTO USE ONLY																				
	\$96.00																					
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(e)].	\$0.00																					
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 20%;">Claims</th> <th style="width: 20%;">Number Filed</th> <th style="width: 20%;">Number Extra</th> <th style="width: 20%;">Rate</th> </tr> </thead> <tbody> <tr> <td>Total Claims</td> <td>18 - 20 =</td> <td>0</td> <td>X \$18.00</td> </tr> <tr> <td>Independent Claims</td> <td>8 - 3 =</td> <td>5</td> <td>X \$78.00</td> </tr> <tr> <td>Multiple dependent claim(s) (if applicable)</td> <td></td> <td>0</td> <td>+ \$260.00</td> </tr> <tr> <td colspan="3" style="text-align: right;">TOTAL OF ABOVE CALCULATIONS =</td> <td>\$486.00</td> </tr> </tbody> </table>	Claims	Number Filed	Number Extra	Rate	Total Claims	18 - 20 =	0	X \$18.00	Independent Claims	8 - 3 =	5	X \$78.00	Multiple dependent claim(s) (if applicable)		0	+ \$260.00	TOTAL OF ABOVE CALCULATIONS =			\$486.00		
Claims	Number Filed	Number Extra	Rate																			
Total Claims	18 - 20 =	0	X \$18.00																			
Independent Claims	8 - 3 =	5	X \$78.00																			
Multiple dependent claim(s) (if applicable)		0	+ \$260.00																			
TOTAL OF ABOVE CALCULATIONS =			\$486.00																			
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28). <div style="text-align: right;">SUBTOTAL =</div>			\$486.00																			
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(f)]. <div style="text-align: right;">TOTAL NATIONAL FEE =</div>			\$486.00																			
Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property. <div style="text-align: right;">TOTAL FEES ENCLOSED =</div>			\$486.00																			
			Amount to be refunded																			
			charged																			

a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 13-2755 in the amount of \$486.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to the Deposit Account No. 13-2755. A duplicate copy of this sheet is enclosed.


NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

MERCK & CO., INC.
 Patent Department, RY60-30
 P.O. Box 2000
 126 East Lincoln Avenue
 Rahway, New Jersey 07065-0970

DATE: AUGUST 23, 2000

PHONE #: (732) 594-6734


 SIGNATURE
 Joseph A. Coppola
 NAME
 38,413
 REGISTRATION NUMBER



08-01-02

DT17 Rec'd PCT/PTO 31 JUL 2002

PATENT
09/622964

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Petrukhin, et al.

Serial No.: 09/622,964 - Case No.: ~~21077YP~~ 201774P

Filed: August 23, 2000

For: BEST'S MACULAR DYSTROPHY GENE

Art Unit:

Examiner:

Assistant Commissioner for Patents
Washington, D.C. 20231

SUBMISSION OF SEQUENCE STATEMENT

Sir:

Please insert the enclosed Sequence Listing in paper and computer readable form into the above-identified application. The Sequence Listing does not contain new matter. The contents of the paper and computer readable forms of the Sequence Listing are the same.

EXPRESS MAIL CERTIFICATE
DATE OF DEPOSIT July 31, 2002
EXPRESS MAIL NO. EF 34109101041015
I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS
BEING DEPOSITED WITH THE UNITED STATES POSTAL
SERVICE AS EXPRESS MAIL "POST OFFICE TO
ADDRESSEE" BEFORE 5 P.M. ON THE ABOVE DATE IN
AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER
FOR PATENTS, WASHINGTON, D.C., 20231.
MAILED BY Christina C. Caffee
DATE 7-31-02

Respectfully submitted,

By Joanne M. Giesser
Joanne M. Giesser
Reg. No. 32,838
Attorney for Applicant

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-3046

Date: July 31, 2002



SEQUENCE LISTING

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Caskey, C. Thomas
Metzker, Michael
Claes, Wadelius

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Gln	Pro	Lys	Asn	Tyr	His	Leu	Pro	Leu	Lys	Asp	Leu	Lys	Thr	Lys	Leu
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His	Leu	Gln	Gln	Arg	Arg	Leu	Asp	Gln	Met	Ser	Thr	Asn	Ile	Gln	Ala
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19

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19

534 Rec'd PCT/PTO 23 AUG 2000

TITLE OF THE INVENTION

BEST'S MACULAR DYSTROPHY GENE

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 This application claims the benefit of U.S. Provisional Application No. 60/122,926, filed December 18, 1998 and U.S. Provisional Application No. 60/075,941, filed February 25, 1998, the contents of which are incorporated herein by reference in their entirety.

10 STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

15

FIELD OF THE INVENTION

The present invention is directed to novel human and mouse DNA sequences encoding a protein which, when present in mutated form, results in the occurrence of Best's Macular Dystrophy.

20

BACKGROUND OF THE INVENTION

- Macular dystrophy is a term applied to a heterogeneous group of diseases that collectively are the cause of severe visual loss in a large number of people. A common characteristic of macular dystrophy is a progressive loss of central vision resulting from the degeneration of the pigmented epithelium underlying the retinal macula. In many forms of macular dystrophy, the end stage of the disease results in legal blindness. More than 20 types of macular dystrophy are known: e.g., age-related macular dystrophy, Stargardt's disease, atypical vitelliform macular dystrophy (VMD1), Usher Syndrome Type 1B, autosomal dominant neovascular inflammatory vitreoretinopathy, familial exudative vitreoretinopathy, and Best's
- 25 macular dystrophy (also known as hereditary macular dystrophy or Best's vitelliform macular dystrophy (VMD2)). For a review of the macular dystrophies, see Sullivan & Daiger, 1996, Mol. Med. Today 2:380-386.
- 30

Best's Macular Dystrophy (BMD) is an inherited autosomal dominant macular dystrophy of unknown biochemical cause. BMD has an age of onset that can range from childhood to after 40. Clinical symptoms include, at early stages, an abnormal accumulation of the yellowish material lipofuscin in the retinal pigmented epithelium (RPE) underlying the macula. This gives rise to a characteristic "egg yolk" appearance of the RPE and gradual loss of visual acuity. With increasing age, the RPE becomes more and more disorganized, as the lipofuscin accumulations disperse and scarring and neovascularization take place. These changes are accompanied by further loss of vision.

The pathological features seen in BMD are in many ways similar to the features seen in age-related macular dystrophy, the leading cause of blindness in older patients in the developed world. Age-related macular dystrophy is an extraordinarily difficult disease to study genetically, since by the time patients are diagnosed, their parents are usually no longer living and their children are still asymptomatic. Thus, family studies which have led to the discovery of the genetic basis of many other diseases have not been practical for age-related macular dystrophy. As there are currently no widely effective treatments for age-related macular dystrophy, it is hoped that study of BMD, and in particular the discovery of the underlying genetic cause of BMD, will shed light on age-related macular dystrophy as well.

Linkage analysis has established that the gene responsible for BMD resides in the pericentric region of chromosome 11, at 11q13, near the markers D11S956, FCER1B, and UGB (Forsman et al., 1992, Clin. Genet. 42:156-159; Hou et al., 1996, Human Heredity 46:211-220). Recently, the gene responsible for BMD was localized to a ~1.7 mB PAC contig lying mostly between the markers D11S1765 and UGB (Cooper et al., 1997, Genomics 41:185-192). Recombination breakpoint mapping in a large Swedish pedigree limited the minimum genetic region containing the BMD gene to a 980 kb interval flanked by the microsatellite markers D11S4076 and UGB (Graff et al., 1997, Hum. Genet. 101: 263-279).

One difficulty in diagnosing BMD is that carriers of the diseased gene for BMD may be asymptomatic in terms of visual acuity and morphological changes of the RPE observable in a routine ophthalmologic examination. There does exist a test, the electro-oculographic examination (EOG), which detects differences in electrical potential between the cornea and the retina, that can distinguish asymptomatic BMD patients from normal individuals. However, the EOG requires

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specialized, expensive equipment, is difficult to administer, and requires that the patient be present at the site of the equipment when the test is performed. It would be valuable to have an alternative method of diagnosing asymptomatic carriers of mutations in the gene responsible for BMD that is simpler, less expensive, and does not require the presence of the patient while the test is being performed. For example, a diagnostic test that relies on a blood sample from a patient suspected of being an asymptomatic carrier of BMD would be ideal.

SUMMARY OF THE INVENTION

The present invention is directed to novel human and mouse DNA sequences that encode the gene CG1CE, which, when mutated, is responsible for Best's macular dystrophy. The present invention includes genomic CG1CE DNA as well as cDNA that encodes the CG1CE protein. The human genomic CG1CE DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The human cDNA encoding CG1CE protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2 or SEQ.ID.NO.:4. The mouse cDNA encoding CG1CE protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:28. Also provided is CG1CE protein encoded by the novel DNA sequences. The human CG1CE protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3 or SEQ.ID.NO.:5. The mouse CG1CE protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:29. Methods of expressing CG1CE protein in recombinant systems are provided. Also provided are diagnostic methods that detect carriers of mutant CG1CE genes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-F shows the genomic DNA sequence of human CG1CE (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon in exon 2 and the stop TAA codon in exon 11 are shown in bold italics. The consensus polyadenylation signal AATAAA in exon 11 is shown in bold. The alternatively spliced part of exon 7 is shown in underlined italics. The exact lengths of two gaps between exons 1 and 2 and between exons 7 and 8 are unknown; these gaps are presented as runs of ten Ns for the sake of convenience. The portion of exon

11 beginning at position 15,788 represents the 3' untranslated region; 132 base pairs downstream of the polyadenylation signal of the CG1CE gene are multiple ESTs, representing the 3'-untranslated region of the ferritin heavy chain gene (FTH). FTH has been mapped to human chromosome 11q13 (Hentze *et al.*, 1986, Proc. Nat. Acad. Sci. 83: 7226-7230); the FTH gene was later shown to be a part of the smallest minimum genetic region containing the BMD gene, as determined by recombination breakpoint mapping in a 12 generation Swedish pedigree (Graff *et al.*, 1997, Hum. Genet. 101: 263-279).

Figure 2 shows the complete sequence of the short form of human CG1CE cDNA (SEQ.ID.NO.:2). The ATG start codon is at position 105; the TAA stop codon is at position 1,860.

Figure 3 shows the complete amino acid sequence of the long form of human CG1CE protein (SEQ.ID.NO.:3). This long form of the human CG1CE protein is produced by translation of the short form of CG1CE cDNA.

Figure 4 shows the complete sequence of the long form of human CG1CE cDNA (SEQ.ID.NO.:4). This long form of the human CG1CE cDNA is produced when an alternative splice donor site is utilized in intron 7. The ATG start codon is at position 105; the TGA stop codon is at position 1410.

Figure 5 shows the complete amino acid sequence of the short form of the human CG1CE protein (SEQ.ID.NO.:5). This short form of the human CG1CE protein is produced by translation of the long form of CG1CE cDNA.

Figure 6 shows the results of sequencing runs of PCR fragments that represent exon 4 and adjacent intronic regions from three individuals from the Swedish pedigree S1, two of whom are affected with BMD. From top to bottom, the runs are: patient S1-5 (homozygous affected with BMD), sense orientation; patient S1-4 (heterozygous affected with BMD), sense orientation; patient S1-3 (normal control, unaffected sister of S1-4), sense orientation; patient S1-5 (affected with BMD), anti-sense orientation; patient S1-4 (affected with BMD), anti-sense orientation; patient S1-3 (normal control), anti-sense orientation. Reading from left to right, the mutation shows up at position 31 of the sequence shown in the case of patients S1-5 and S1-4. The mutation in family S1 changes tryptophan to cysteine.

Figure 7 shows a multiple sequence alignment of human CG1CE protein with partial sequences of related proteins from *C. elegans*. Related proteins from *C. elegans* were identified by BLASTP analysis of non-redundant GenBank

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database. This figure shows that two amino acids mutated in two different Swedish families with BMD (families S1 and SL76) are evolutionarily conserved. 15 of 16 related proteins from *C. elegans* contain a tryptophan at the position of the mutation in family S1, as does the wild-type CG1CE gene. Only one *C. elegans* protein does not have a tryptophan at the position of the mutation. In this protein (accession number p34577), tryptophan is changed for isofunctional phenylalanine (phenylalanine is highly similar to tryptophan in that it also is a hydrophobic aromatic amino acid). Mutation in the BMD family SL76 changes a tyrosine to histidine. Again, all 16 related proteins from *C. elegans* contain tyrosine or isofunctional phenylalanine in this position (tyrosine is highly similar to phenylalanine in that it also is an aromatic amino acid).

Figure 8A-C shows the complete sequence of mouse CG1CE cDNA (SEQ.ID.NO.:28) and mouse CG1CE protein (SEQ.ID.NO.:29).

Figure 9A-B shows an alignment of the amino acid sequences of the long form of human CG1CE protein (SEQ.ID.NO.:3) and mouse CG1CE protein (SEQ.ID.NO.:29). In this figure, CG1CE is referred to as "bestrophin."

Figure 10A-C shows the results of *in situ* hybridization experiments demonstrating that mouse CG1CE mRNA expression is localized to the retinal pigmented epithelium cells (RPE). Figure 10A shows the results of using an antisense CG1CE probe. The antisense probe hybridizes to mouse CG1CE mRNA present in the various cell layers of the retina, labeling with dark bands the cells containing CG1CE mRNA. The antisense probe strongly hybridized to the RPE cells and not to the cells of the other layers of the retina. Figure 10B shows the results using a sense CG1CE probe as a control. The sense probe does not hybridize to CG1CE mRNA and does not label the RPE cells. Figure 10C is a higher magnification of the RPE cells from Figure 10A. Human CG1CE mRNA shows a similar distribution, being confined to the RPE cells of the human retina.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a CG1CE protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than

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5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- CG1CE proteins. Whether a given CG1CE protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

“Substantially free from other nucleic acids” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, a CG1CE DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- CG1CE nucleic acids. Whether a given CG1CE DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

A “conservative amino acid substitution” refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

The present invention relates to the identification and cloning of CG1CE, a gene which, when mutated, is responsible for Best’s macular dystrophy. That CG1CE is the Best’s macular dystrophy gene is supported by various observations:

1. CG1CE maps to the genetically defined region of human chromosome 11q12-q13 that has been shown to contain the Best’s macular dystrophy gene. CG1CE is present on two PAC clones, 759J12 and 466A11, that lie precisely in the most narrowly defined region that has been shown to contain CG1CE (Cooper *et al.*, 1997, *Genomics* 41:185-192; Stöhr *et al.*, 1997, *Genome Res.* 8:48-56; Graff *et al.*, 1997, *Hum. Genet.* 101: 263-279).

2. CG1CE is expressed predominately in the retina.

3. In patients having Best's macular dystrophy, CG1CE contains mutations in evolutionarily conserved amino acids.

4. The CG1CE genomic clones contain another gene (FTH) that has been physically associated with the Best's macular dystrophy region (Cooper *et al.*, 1997, Genomics 41:185-192; Stöhr *et al.*, 1997, Genome Res. 8:48-56; Graff *et al.*, 1997, Hum. Genet. 101:263-279). The FTH and CG1CE genes are oriented tail-to-tail; the distance between their polyadenylation signals is 132 bp.

The present invention provides DNA encoding CG1CE that is substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding CG1CE. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 11 exons. These exons collectively have an open reading frame that encodes a protein of 585 amino acids. If an alternative splice donor site is utilized in exon 7, a cDNA containing an additional 203 bases is produced. Although longer, this cDNA contains a shorter open reading frame of 1,305 bases (due to the presence of a change in reading frame that introduces a stop codon) that encodes a protein of 435 amino acids. Thus, the present invention includes two cDNA molecules encoding two forms of CG1CE protein that are substantially free from other nucleic acids and have the nucleotide sequences shown in Figure 2 as SEQ.ID.NO.:2 and in Figure 4 as SEQ.ID.NO.:4.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding regions of SEQ.ID.NO.:2 and SEQ.ID.NO.:4. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 105-1,859 of SEQ.ID.NO.:2 and positions 105-1,409 of SEQ.ID.NO.:4. Also included are recombinant DNA molecules having a nucleotide sequence comprising positions 105-1,859 of SEQ.ID.NO.:2 and positions 105-1,409 of SEQ.ID.NO.:4.

Portions of the cDNA sequences of SEQ.ID.NO.:2 and SEQ.ID.NO.:4 are found in two retina-specific ESTs deposited in GenBank by The Institute for Genomic Research (accession numbers AA318352 and AA317489). Other ESTs that correspond to this cDNA are accession numbers AA307119 (from a colon carcinoma), AA205892 (from neuronal cell line), and AA326727 (from human

cerebellum). A true mouse ortholog of the CG1CE gene is represented in the mouse EST AA497726 (from mouse testis).

The novel DNA sequences of the present invention encoding CG1CE, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which CG1CE is not naturally linked, to form "recombinant DNA molecules" encoding CG1CE. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NOs.:1, 2, or 4 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

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The present invention includes a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position 7,233 of SEQ.ID.NO.:1 is C, A, or G rather than T, so that the codon at positions 7,233-7,235 does not encode tyrosine. Also included in the present invention is a
5 DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that at least one of the nucleotides at position 7,234 or 7,235 has been changed so that the codon at positions 7,233-7,235 does not encode tyrosine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the
10 nucleotide at position 357 is C, A, or G rather than T, so that the codon at positions 357-359 does not encode tyrosine. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that at least one of the nucleotides at position 358 or 359 has been changed so that the codon at positions 357-359 does not encode tyrosine.

15 The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that the nucleotide at position 357 is C, A, or G rather than T, so that the codon at positions 357-359 does not encode tyrosine. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,409 of
20 SEQ.ID.NO.:4 except that at least one of the nucleotides at position 358 or 359 has been changed so that the codon at positions 357-359 does not encode tyrosine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position 3,330 is C rather than A. Also included in the present invention is a DNA molecule
25 having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position 3,330 of SEQ.ID.NO.:1 is G, C, or T rather than A, so that the codon at positions 3,330-3,332 does not encode threonine. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that at least one of the nucleotides at position 3,330 or 3,331
30 has been changed so that the codon at positions 3,330-3,332 does not encode threonine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the nucleotide at position 120 is C rather than A. Also included in the present invention

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is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the nucleotide at position 120 is G, C, or T rather than A, so that the codon at positions 120-122 does not encode threonine. Also included in the present invention is a DNA molecule having a nucleotide sequence
5 that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that at least one of the nucleotides at position 120 or 121 has been changed so that the codon at positions 120-122 does not encode threonine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that the
10 nucleotide at position 120 is C rather than A. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that the nucleotide at position 120 is G, C, or T rather than A, so that the codon at positions 120-122 does not encode threonine. Also included in the present invention is a DNA molecule having a nucleotide sequence
15 that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that at least one of the nucleotides at position 120 or 121 has been changed so that the codon at positions 120-122 does not encode threonine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position
20 8,939 is A rather than T. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position 8,939 of SEQ.ID.NO.:1 is A, G, or C, rather than T, so that the codon at positions 8,939-8,941 does not encode tyrosine. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to
25 SEQ.ID.NO.:1 except that at least one of the nucleotides at position 8,939-8,941 has been changed so that the codon at positions 8,939-8,941 does not encode tyrosine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the nucleotide at position 783 is A rather than T. Also included in the present invention
30 is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the nucleotide at position 783 is A, G, or C rather than T so that the codon at positions 783-785 does not encode tyrosine. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that at least one of the

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nucleotides at position 783-785 has been changed so that the codon at positions 783-785 does not encode tyrosine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that the
5 nucleotide at position 783 is A rather than T. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that the nucleotide at position 783 is A, G, or C rather than T, so that the codon at positions 783-785 does not encode tyrosine. Also included in the present invention is a DNA molecule having a nucleotide sequence
10 that is identical to positions 105-1,409 of SEQ.ID.NO.:4 except that at least one of the nucleotides at position 783-785 has been changed so that the codon at positions 783-785 does not encode tyrosine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position
15 11,241 is A rather than G. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to SEQ.ID.NO.:1 except that the nucleotide at position 11,241 is A, C, or T, rather than G, so that the codon at positions 11,240-11,242 does not encode glycine. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to
20 SEQ.ID.NO.:1 except that at least one of the nucleotides at position 11,240 or 11,241 has been changed so that the codon at positions 11,240-11,242 does not encode glycine.

The present invention includes a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the
25 nucleotide at position 1,000 is A rather than G. Also included in the present invention is a DNA molecule having a nucleotide sequence that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that the nucleotide at position 1,000 is A, C, or T rather than G, so that the codon at positions 999-1,001 does not encode glycine. Also included in the present invention is a DNA molecule having a nucleotide sequence
30 that is identical to positions 105-1,859 of SEQ.ID.NO.:2 except that at least one of the nucleotides at position 999 or 1,000 has been changed so that the codon at positions 999-1,001 does not encode glycine.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding CGICE protein.

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Such recombinant host cells can be cultured under suitable conditions to produce CGICE protein. An expression vector containing DNA encoding CGICE protein can be used for expression of CGICE protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of CGICE protein and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

A variety of mammalian expression vectors can be used to express recombinant CGICE in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNA1 and pcDNA1amp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, CGICE can be purified by conventional techniques to a level that is substantially free from other proteins.

The present invention includes CGICE protein substantially free from other proteins. The amino acid sequence of the full-length CGICE protein is shown in Figure 3 as SEQ.ID.NO.:3. Thus, the present invention includes CGICE protein substantially free from other proteins having the amino acid sequence SEQ.ID.NO.:3. Also included in the present invention is a CGICE protein that is produced from an alternatively spliced CGICE mRNA where the protein has the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5.

Mutated forms of CGICE proteins are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NOs.:3 and 5 that give rise to Best's macular dystrophy are within the scope of the present invention. Accordingly, the present invention includes a protein having the amino acid sequence

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shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 93 is cysteine rather than tryptophan. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 93 is cysteine rather than tryptophan. The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 93 is not tryptophan. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 93 is not tryptophan.

The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 85 is histidine rather than tyrosine. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 85 is histidine rather than tyrosine. The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 85 is not tyrosine. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 85 is not tyrosine.

The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 6 is proline rather than threonine. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 6 is proline rather than threonine. The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 6 is not threonine. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 6 is not threonine.

The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 227 is asparagine rather than tyrosine. The present invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 227 is asparagine rather than tyrosine. The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 227 is not tyrosine. The present

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invention also includes a protein having the amino acid sequence shown in Figure 5 as SEQ.ID.NO.:5 except that the amino acid at position 227 is not tyrosine.

The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 299 is glutamate rather than glycine. The present invention includes a protein having the amino acid sequence shown in Figure 3 as SEQ.ID.NO.:3 except that the amino acid at position 299 is not glycine. As with many proteins, it is possible to modify many of the amino acids of CG1CE and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified CG1CE proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as CG1CE. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NOs.:3 or 5 wherein the polypeptides still retain substantially the same biological activity as CG1CE. The present invention also includes polypeptides where two amino acid substitutions have been made in SEQ.ID.NOs.:3 or 5 wherein the polypeptides still retain substantially the same biological activity as CG1CE. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in CG1CE is also present in one of the *C. elegans* proteins whose partial sequence is shown in Figure 7.

The CG1CE proteins of the present invention may contain post-translational modifications, *e.g.*, covalently linked carbohydrate.

The present invention also includes chimeric CG1CE proteins. Chimeric CG1CE proteins consist of a contiguous polypeptide sequence of at least a portion of a CG1CE protein fused to a polypeptide sequence of a non- CG1CE protein.

The present invention also includes isolated forms of CG1CE proteins and CG1CE DNA. By "isolated CG1CE protein" or "isolated CG1CE DNA" is meant CG1CE protein or DNA encoding CG1CE protein that has been isolated from

a natural source. Use of the term "isolated" indicates that CG1CE protein or CG1CE DNA has been removed from its normal cellular environment. Thus, an isolated CG1CE protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply
5 that an isolated CG1CE protein is the only protein present, but instead means that an isolated CG1CE protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the CG1CE protein. Thus, a CG1CE protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is
10 an "isolated CG1CE protein."

A cDNA fragment encoding full-length CG1CE can be isolated from a human retinal cell cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for CG1CE shown in Figure 2 as SEQ.ID.NO.:2 or in Figure 4 as
15 SEQ.ID.NO.:4. Suitable primer pairs would be, *e.g.*:

CAGGGAGTCCCACCAGCC (SEQ.ID.NO.:6) and
TCCCCATTAGGAAGCAGG (SEQ.ID.NO.:7)
for SEQ.ID.NO.:2; and
CAGGGAGTCCCACCAGCC (SEQ.ID.NO.:6) and
20 TCTCCTCTTTGTTTCAGGC (SEQ.ID.NO.:8)
for SEQ.ID.NO.:4.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM
25 MgCl₂, 200 μ M for each dNTP, 50 mM KCl, 0.2 μ M for each primer, 10 ng of DNA template, 0.05 units/ μ l of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W.
30 Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding CG1CE can be isolated would be Human Retina 5'-stretch cDNA library in lambda gt10 or lambda

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gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of
 5 585 amino acids (SEQ.ID.NO.:3) or an open reading frame of 435 amino acids
 (SEQ.ID.NO.:5) can be obtained. This cDNA fragment can be cloned into a suitable
 cloning vector or expression vector. For example, the fragment can be cloned into the
 mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, Ca). CG1CE
 protein can then be produced by transferring an expression vector encoding CG1CE
 10 or portions thereof into a suitable host cell and growing the host cell under
 appropriate conditions. CG1CE protein can then be isolated by methods well known
 in the art.

As an alternative to the above-described PCR method, a cDNA clone
 encoding CG1CE can be isolated from a cDNA library using as a probe
 15 oligonucleotides specific for CG1CE and methods well known in the art for screening
 cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*,
 Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring
 Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA
 Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II.
 20 Oligonucleotides that are specific for CG1CE and that can be used to screen cDNA
 libraries can be readily designed based upon the cDNA sequence of CG1CE shown in
 Figure 2 as SEQ.ID.NO.:2 or in Figure 4 as SEQ.ID.NO.:4 and can be synthesized by
 methods well-known in the art.

Genomic clones containing the CG1CE gene can be obtained from
 25 commercially available human PAC or BAC libraries available from Research
 Genetics, Huntsville, AL. PAC clones containing the CG1CE gene (*e.g.*, PAC
 759J12, PAC 466A11) are commercially available from Research Genetics,
 Huntsville, AL (Catalog number for individual PAC clones is RPCI.C).
 Alternatively, one may prepare genomic libraries, especially in P1 artificial
 30 chromosome vectors, from which genomic clones containing the CG1CE can be
 isolated, using probes based upon the CG1CE sequences disclosed herein. Methods
 of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.*
 6:84-89).

The novel DNA sequences of the present invention can be used in various diagnostic methods relating to Best's macular dystrophy. The present invention provides diagnostic methods for determining whether a patient carries a mutation in the CG1CE gene that predisposes that patient toward the development of Best's macular dystrophy. In broad terms, such methods comprise determining the DNA sequence of a region of the CG1CE gene from the patient and comparing that sequence to the sequence from the corresponding region of the CG1CE gene from a normal person, *i.e.*, a person who does not suffer from Best's macular dystrophy.

Such methods of diagnosis may be carried out in a variety of ways.

For example, one embodiment comprises:

- (a) providing PCR primers from a region of the CG1CE gene where it is suspected that a patient harbors a mutation in the CG1CE gene;
- (b) performing PCR on a DNA sample from the patient to produce a PCR fragment from the patient;
- (c) performing PCR on a control DNA sample having a nucleotide sequence selected from the group consisting of SEQ.ID.NOs.:1, 2 and SEQ.ID.NO.:4 to produce a control PCR fragment;
- (d) determining the nucleotide sequence of the PCR fragment from the patient and the nucleotide sequence of the control PCR fragment;
- (e) comparing the nucleotide sequence of the PCR fragment from the patient to the nucleotide sequence of the control PCR fragment; where a difference between the nucleotide sequence of the PCR fragment from the patient and the nucleotide sequence of the control PCR fragment indicates that the patient has a mutation in the CG1CE gene.

In a particular embodiment, the PCR primers are from the coding region of the CG1CE gene, *i.e.*, from the coding region of SEQ.ID.NOs.:1, 2, or 4.

In a particular embodiment, the DNA sample from the patient is cDNA that has been prepared from an RNA sample from the patient. In another embodiment, the DNA sample from the patient is genomic DNA.

In a particular embodiment, the nucleotide sequences of the PCR fragment from the patient and the control PCR fragment are determined by DNA sequencing.

In a particular embodiment, the nucleotide sequences of the PCR fragment from the patient and the control PCR fragment are compared by direct

comparison after DNA sequencing. In another embodiment, the comparison is made by a process that includes hybridizing the PCR fragment from the patient and the control PCR fragment and then using an endonuclease that cleaves at any mismatched positions in the hybrid but does not cleave the hybrid if the two fragments match perfectly. Such an endonuclease is, *e.g.*, S1. In this embodiment, the conversion of the PCR fragment from the patient to smaller fragments after endonuclease treatment indicates that the patient carries a mutation in the CG1CE gene. In such embodiments, it may be advantageous to label (radioactively, enzymatically, immunologically, *etc.*) the PCR fragment from the patient or the control PCR fragment.

The present invention provides a method of diagnosing whether a patient carries a mutation in the CG1CE gene that comprises:

- (a) obtaining an RNA sample from the patient;
- (b) performing reverse transcription-PCR (RT-PCR) on the RNA sample using primers that span a region of the coding sequence of the CG1CE gene to produce a PCR fragment from the patient where the PCR fragment from the patient has a defined length, the length being dependent upon the identity of the primers that were used in the RT-PCR;
- (c) hybridizing the PCR fragment to DNA having a sequence selected from the group consisting of SEQ.ID.NO.:1, 2 and SEQ.ID.NO.:4 to form a hybrid ;
- (d) treating the hybrid produced in step (c) with an endonuclease that cleaves at any mismatched positions in the hybrid but does not cleave the hybrid if the two fragments match perfectly;
- (e) determining whether the endonuclease cleaved the hybrid by determining the length of the PCR fragment from the patient after endonuclease treatment where a reduction in the length of the PCR fragment from the patient after endonuclease treatment indicates that the patient carries a mutation in the CG1CE gene.

The present invention provides a method of diagnosing whether a patient carries a mutation in the CG1CE gene that comprises:

- (a) making cDNA from an RNA sample from the patient;
- (b) providing a set of PCR primers based upon SEQ.ID.NO.:2 or SEQ.ID.NO.:4;

(c) performing PCR on the cDNA to produce a PCR fragment from the patient;

(d) determining the nucleotide sequence of the PCR fragment from the patient;

5 (e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:2 or SEQ.ID.NO.:4; where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:2 or SEQ.ID.NO.:4 indicates that the patient carries a mutation in the CGICE gene.

10 The present invention provides a method of diagnosing whether a patient carries a mutation in the CGICE gene that comprises:

(a) preparing genomic DNA from the patient;

(b) providing a set of PCR primers based upon SEQ.ID.NO.:1, SEQ.ID.NO.:2, or SEQ.ID.NO.:4;

15 (c) performing PCR on the genomic DNA to produce a PCR fragment from the patient;

(d) determining the nucleotide sequence of the PCR fragment from the patient;

(e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:2 or SEQ.ID.NO.:4; where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:2 or SEQ.ID.NO.:4 indicates that the patient carries a mutation in the CGICE gene.

25 In a particular embodiment, the primers are selected so that they amplify a portion of SEQ.ID.NOs.:2 or 4 that includes at least one position selected from the group consisting of: positions 120, 121, 122, 357, 358, 359, 381, 382, 383, 783, 784, and 785. In another embodiment, the primers are selected so that they amplify a portion of SEQ.ID.NOs.:2 or 4 that includes at least one position selected from the group consisting of: positions 384, 385, and 386. In another embodiment,

30 the primers are selected so that they amplify a portion of SEQ.ID.NO.:2 that includes at least one position selected from the group consisting of: positions 999, 1,000, and 1,001. In another embodiment, the primers are selected so that they amplify a portion of SEQ.ID.NOs.:2 or 4 that includes at least one codon that encodes an amino acid

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present in CGICE that is also present in the corresponding position in at least one of the *C. elegans* proteins whose partial amino acid sequence is shown in Figure 7.

In a particular embodiment, the present invention provides a diagnostic method for determining whether a person carries a mutation of the CGICE gene in which the G at position 383 of SEQ.ID.NO.:2 has been changed to a C. This change results in the creation of a Fnu4HI restriction site. By amplifying a PCR fragment spanning position 383 of SEQ.ID.NO.:2 from DNA or cDNA prepared from a person, digesting the PCR fragment with Fnu4HI, and visualizing the digestion products, *e.g.*, by SDS-PAGE, one can easily determine if the person carries the G383C mutation. For example, one could use the PCR primer pair 5'-CTCCTGCCCAGGCTTCTAC-3' (SEQ.ID.NO.:30) and 5'-CTTGCTCTGCCTTGCCTTC-3' (SEQ.ID.NO.:31) to amplify a 125 base pair fragment. Heterozygotes for the G383C mutation have three Fnu4HI digestion products: 125 bp, 85 bp, and 40 bp; homozygotes have two: 85 bp and 40 bp; and wild-type individuals have a single fragment of 125 bp.

In a particular embodiment, the present invention provides a diagnostic method for determining whether a person carries a mutation of the CGICE gene in which the T at position 783 of SEQ.ID.NO.:2 has been changed to an A. This change results in the creation of a PflMI restriction site. By amplifying a PCR fragment spanning position 783 of SEQ.ID.NO.:2 from DNA or cDNA prepared from a person, digesting the PCR fragment with PflMI, and visualizing the digestion products, *e.g.*, by SDS-PAGE, one can easily determine if the person carries the T783A mutation.

The present invention also provides oligonucleotide probes, based upon the sequences of SEQ.ID.NOs.:1, 2, or 4, that can be used in diagnostic methods related to Best's macular dystrophy. In particular, the present invention includes DNA oligonucleotides comprising at least 18 contiguous nucleotides of at least one of a sequence selected from the group consisting of: SEQ.ID.NOs.:1, 2 and SEQ.ID.:NO.4. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide probes can be packaged in kits.

In addition to the diagnostic utilities described above, the present invention makes possible the recombinant expression of the CGICE protein in various cell types. Such recombinant expression makes possible the study of this protein so that its biochemical activity and its role in Best's macular dystrophy can be elucidated.

The present invention also makes possible the development of assays which measure the biological activity of the CG1CE protein. Such assays using recombinantly expressed CG1CE protein are especially of interest. Assays for CG1CE protein activity can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of CG1CE protein. Such identified compounds can serve as "leads" for the development of pharmaceuticals that can be used to treat patients having Best's macular dystrophy. In versions of the above-described assays, mutant CG1CE proteins are used and inhibitors or activators of the activity of the mutant CG1CE proteins are discovered.

Such assays comprise:

- (a) recombinantly expressing CG1CE protein or mutant CG1CE protein in a host cell;
 - (b) measuring the biological activity of CG1CE protein or mutant CG1CE protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CG1CE protein or mutant CG1CE protein;
- where a change in the biological activity of the CG1CE protein or the mutant CG1CE protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CG1CE protein or mutant CG1CE protein.

The present invention also includes antibodies to the CG1CE protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire CG1CE protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, CG1CE protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

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For the production of monoclonal antibodies, CG1CE protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells
5 are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce CG1CE polypeptides into the
10 cells of target organs, e.g., the pigmented epithelium of the retina or other parts of the retina. Nucleotides encoding CG1CE polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding
15 CG1CE polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with CG1CE polypeptides will be particularly useful for the treatment of
20 diseases where it is beneficial to elevate CG1CE activity.

The present invention includes DNA comprising nucleotides encoding mouse CG1CE. Included within such DNA is the DNA sequence shown in Figure 8A-C (SEQ. ID. NO.:28). Also included is DNA comprising positions 11-1,663 of SEQ. ID. NO.:28. Also included are mutant versions of DNA encoding mouse
25 CG1CE. Included is DNA comprising nucleotides that are identical to positions 11-1,663 of SEQ. ID. NO.:28 except that at least one of the nucleotides at positions 26-28, positions 263-265, positions 287-289, positions 689-691, and/or positions 905-907 differs from the corresponding nucleotide at positions 26-28, positions 263-265, positions 287-289, positions 689-691, and/or positions 905-907 of SEQ. ID. NO.:28.
30 Particularly preferred versions of mutant DNAs are those in which the nucleotide change results in a change in the corresponding encoded amino acid. The DNA encoding mouse CG1CE can be in isolated form, can be substantially free from other nucleic acids, and/or can be recombinant DNA.

The present invention includes mouse CG1CE protein (SEQ. ID. NO.:29). This mouse CG1CE protein can be in isolated form and/or can be substantially free from other proteins. Mutant versions of mouse CG1CE protein are also part of the present invention. Examples of such mutant mouse CG1CE proteins are proteins that are identical to SEQ. ID. NO.:29 except that the amino acid at position 6, position 85, position 93, position 227, and/or position 299 differs from the corresponding amino acid at position 6, position 85, position 93, position 227, and/or position 299 in SEQ. ID. NO.:29.

cDNA encoding mouse CG1CE can be amplified by PCR from cDNA libraries made from mouse eye or mouse testis. Suitable primers can be readily designed based upon SEQ. ID. NO.:28. Alternatively, cDNA encoding mouse CG1CE can be isolated from cDNA libraries made from mouse eye or mouse testis by the use of oligonucleotide probes based upon SEQ. ID. NO.:28.

In situ hybridization studies demonstrated that mouse CG1CE is specifically expressed in the retinal pigmented epithelium (see Figure 10).

By providing DNA encoding mouse CG1CE, the present invention allows for the generation of an animal model of Best's macular dystrophy. This animal model can be generated by making "knockout" or "knockin" mice containing altered CG1CE genes. Knockout mice can be generated in which portions of the mouse CG1CE gene have been deleted. Knockin mice can be generated in which mutations that have been shown to lead to Best's macular dystrophy when present in the human CG1CE gene are introduced into the mouse gene. In particular, mutations resulting in changes in amino acids 6, 85, 93, 227, or 299 of the mouse CG1CE protein (SEQ.ID.NO.:29) are contemplated. Such knockout and knockin mice will be valuable tools in the study of the Best's macular dystrophy disease process and will provide important model systems in which to test potential pharmaceuticals or treatments for Best's macular dystrophy.

Methods of producing knockout and knockin mice are well known in the art. For example, the use of gene-targeted ES cells in the generation of gene-targeted transgenic knockout mice is described in, *e.g.*, Thomas et al., 1987, Cell 51:503-512, and is reviewed elsewhere (Frohman et al., 1989, Cell 56:145-147; Capecchi, 1989, Trends in Genet. 5:70-76; Baribault et al., 1989, Mol. Biol. Med. 6:481-492).

Techniques are available to inactivate or alter any genetic region to virtually any mutation desired by using targeted homologous recombination to insert specific changes into chromosomal genes. Generally, use is made of a “targeting vector,” *i.e.*, a plasmid containing part of the genetic region it is desired to mutate. By virtue of the homology between this part of the genetic region on the plasmid and the corresponding genetic region on the chromosome, homologous recombination can be used to insert the plasmid into the genetic region, thus disrupting the genetic region. Usually, the targeting vector contains a selectable marker gene as well.

In comparison with homologous extrachromosomal recombination, which occurs at frequencies approaching 100%, homologous plasmid-chromosome recombination was originally reported to only be detected at frequencies between 10^{-6} and 10^{-3} (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395; Smithies et al., 1985, Nature 317: 230-234; Thomas et al., 1986, Cell 44:419-428). Nonhomologous plasmid-chromosome interactions are more frequent, occurring at levels 10^5 -fold (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395) to 10^2 -fold (Thomas et al., 1986, Cell 44:419-428) greater than comparable homologous insertion.

To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting homologous alteration events uses the polymerase chain reaction (PCR) to screen pools of transformant cells for homologous insertion, followed by screening individual clones (Kim et al., 1988, Nucleic Acids Res. 16:8887-8903; Kim et al., 1991, Gene 103:227-233). Alternatively, a positive genetic selection approach has been developed in which a marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly (Sedivy et al., 1989, Proc. Natl. Acad. Sci. USA 86:227-231). One of the most powerful approaches developed for selecting homologous recombinants is the positive-negative selection (PNS) method developed for genes for which no direct selection of the alteration exists (Mansour et al., 1988, Nature 336:348-352; Capecchi, 1989, Science 244:1288-1292; Capecchi, 1989, Trends in Genet. 5:70-76). The PNS method is more efficient for targeting genes which are not expressed at high levels because the marker gene has its own promoter. Nonhomologous recombinants are selected against by using the Herpes Simplex virus thymidine kinase (HSV-TK) gene and selecting against its

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nonhomologous insertion with herpes drugs such as gancyclovir (GANC) or FIAU (1-(2-deoxy 2-fluoro-B-D-arabinofluranosyl)-5-iodouracil). By this counter-selection, the percentage of homologous recombinants in the surviving transformants can be increased.

5

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

10 Identification of the human CGICE gene and cDNA cloning

Construction of Libraries for Shotgun Sequencing

Bacterial strains containing the BMD PACs (P1 Artificial Chromosomes) were received from Research Genetics (Huntsville, AL). The minimum tiling path between markers D11S4076 and UGB that represents the
 15 minimum genetic region containing the BMD gene includes the following nine PAC clones: 363M5 (140 kb), 519O13(120 kb), 527E4 (150 kb), 688P12 (140 kb), 741N15 (170 kb), 756B9 (120 kb), 759J12 (140 kb), 1079D9 (170 kb), and 363P2 (160 kb). Cells were streaked on Luria-Bertani (LB) agar plates supplemented with the appropriate antibiotic. A single colony was picked up and subjected to colony-
 20 PCR analysis with corresponding STS primers described in Cooper *et al.*, 1997, Genomics 41:185-192 to confirm the authenticity of PAC clones. A single positive colony was used to prepare a 5-ml starter culture and then 1-L overnight culture in LB medium. The cells were pelleted by centrifugation and PAC DNA was purified by equilibrium centrifugation in cesium chloride-ethidium bromide gradient (Sambrook,
 25 Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press). Purified PAC DNA was brought to 50 mM Tris pH 8.0, 15 mM MgCl₂, and 25% glycerol in a volume of 2 ml and placed in a AERO-MIST nebulizer (CIS-US, Bedford, MA). The nebulizer was attached to a nitrogen gas source and the DNA was randomly sheared at 10 psi for 30 sec. The
 30 sheared DNA was ethanol precipitated and resuspended in TE (10 mM Tris, 1 mM EDTA). The ends were made blunt by treatment with Mung Bean Nuclease (Promega, Madison, WI) at 30°C for 30 min, followed by phenol/chloroform

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extraction, and treatment with T4 DNA polymerase (GIBCO/BRL, Gaithersburg, MD) in multicore buffer (Promega, Madison, WI) in the presence of 40 uM dNTPs at 16°C. To facilitate subcloning of the DNA fragments, BstX I adapters (Invitrogen, Carlsbad, CA) were ligated to the fragments at 14°C overnight with T4 DNA ligase (Promega, Madison, WI). Adapters and DNA fragments less than 500 bp were removed by column chromatography using a cDNA sizing column (GIBCO/BRL, Gaithersburg, MD) according to the instructions provided by the manufacturer. Fractions containing DNA greater than 1 kb were pooled and concentrated by ethanol precipitation. The DNA fragments containing BstX I adapters were ligated into the BstX I sites of pSHOT II which was constructed by subcloning the BstX I sites from pcDNA II (Invitrogen, Carlsbad, CA) into the BssH II sites of pBlueScript (Stratagene, La Jolla, CA). pSHOT II was prepared by digestion with BstX I restriction endonuclease and purified by agarose gel electrophoresis. The gel purified vector DNA was extracted from the agarose by following the Prep-A-Gene (BioRad, Richmond, CA) protocol. To reduce ligation of the vector to itself, the digested vector was treated with calf intestinal phosphatase (GIBCO/BRL, Gaithersburg, MD). Ligation reactions of the DNA fragments with the cloning vector were transformed into ultra-competent XL-2 Blue cells (Stratagene, La Jolla, CA), and plated on LB agar plates supplemented with 100 µg/ml ampicillin. Individual colonies were picked into a 96 well plate containing 100 µl/well of LB broth supplemented with ampicillin and grown overnight at 37°C. Approximately 25 µl of 80% sterile glycerol was added to each well and the cultures stored at -80°C.

Preparation of plasmid DNA

Glycerol stocks were used to inoculate 5 ml of LB broth supplemented with 100 µg/ml ampicillin either manually or by using a Tecan Genesis RSP 150 robot (Tecan AG, Hombrechtikon, Switzerland) programmed to inoculate 96 tubes containing 5 ml broth from the 96 wells. The cultures were grown overnight at 37°C with shaking to provide aeration. Bacterial cells were pelleted by centrifugation, the supernatant decanted, and the cell pellet stored at -20°C. Plasmid DNA was prepared with a QIAGEN Bio Robot 9600 (QIAGEN, Chatsworth, CA) according to the Qiawell Ultra protocol. To test the frequency and size of inserts, plasmid DNA was digested with the restriction endonuclease Pvu II. The size of the restriction

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endonuclease products was examined by agarose gel electrophoresis with the average insert size being 1 to 2 kb.

DNA Sequence Analysis of Shotgun clones

5 DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS (Perkin Elmer, Norwalk, CT). DNA sequence analysis was performed with M13 forward and reverse primers. Following amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated
10 sequencer (Perkin Elmer, Norwalk, CT). Approximately 4 sequencing reactions were performed per kb of DNA to be examined (384 sequencing reactions per each of nine PACs).

Assembly of DNA sequences

15 Phred/Phrap was used for DNA sequences assembly. This program was developed by Dr. Phil Green and licensed from the University of Washington (Seattle, WA). Phred/Phrap consists of the following programs: Phred for base-calling, Phrap for sequence assembly, Crossmatch for sequence comparisons, Consed and Phrapview for visualization of data, Repeatmasker for screening repetitive
20 sequences. Vector and *E. coli* DNA sequences were identified by Crossmatch and removed from the DNA sequence assembly process. DNA sequence assembly was on a SUN Enterprise 4000 server running a Solaris 2.51 operating system (Sun Microsystems Inc., Mountain View, CA) using default Phrap parameters. The sequence assemblies were further analyzed using Consed and Phrapview.

25

Identification of new microsatellite genetic markers from the Best's macular dystrophy region

Isolation of CA microsatellites from PAC-specific sublibraries,
Southern blotting and hybridization of PAC DNA with a (dC-dA)_n(dG-dT)_n probe
30 (Pharmacia Biotech, Uppsala, Sweden) was used to confirm the presence of CA repeats in nine PAC clones that represent a minimum tiling path. Shotgun PAC-specific sublibraries were constructed from DNA of all 9 PAC clones using a protocol described above. The sublibraries were plated on agar plates, and colonies were transferred to nylon membranes and probed with randomly primed polynucleotide,

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(dC-dA)_n (dG-dT)_n, Hybridization was performed overnight in a solution containing 6X SSC, 20 mM sodium phosphate buffer (pH 7.0), 1% bovine serum albumin, and 0.2% sodium dodecyl sulfate at 65°C. Filters were washed four times for 15 min each in 2X SSC and 0.2% SDS at 65°C. CA-positive subclones were identified for all but one PAC clone (527E4). DNA from these subclones was isolated and sequenced as described above for the shotgun library clones.

Identification of simple repeat sequences in assembled DNA sequences. DNA sequence at the final stage of assembly was checked for the presence of microsatellite repeats using a Consed visualization tool of the Phred/Phrap package.

Polymorphism analysis and recombination mapping

Sequence fragments containing CA repeats were analyzed using the PRIMER program; oligonucleotide pairs flanking each of the CA repeats were synthesized. The forward primer was kinase-labeled with [gamma-³²P]-ATP. Amplification of the genomic DNA was performed in a total volume of 10 µl containing 5 ng/µl of genomic DNA; 10 mM Tris-HCl pH 8.3; 1.5 mM MgCl₂; 50 mM KCl; 0.01% gelatin; 200 µM dNTPs; 0.2 pmol/µl of both primers; 0.025 unit/µl of Taq polymerase. The PCR program consisted of 94°C for 3 min followed by 30 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 2 min and a final elongation step at 72°C for 10 min. Following amplification, samples were mixed with 2 vol of a formamide dye solution and run on a 6% polyacrylamide sequencing gel. Two newly identified markers detected two recombination events in disease chromosomes of individuals from family S1. This limited the minimum genetic region to the interval covered by 6 PAC clones: 519O13, 759J12, 756B9, 363M5, 363P2, and 741N15.

Identification of the retina-specific EST hit in the pCA759J12-2 clone.

A CA-positive subclone (pCA759J12-2) was identified in the shotgun library generated from the PAC 759j12 DNA by hybridization to the (dC-dA)_n (dG-dT)_n probe. DNA sequence from pCA759J12-2 was queried against the EST sequences in the GenBank database using the BLAST algorithm (S.F. Altschul, *et al.*, 1990, J. Mol. Biol. 215:403-410). The BLAST analysis identified a high degree of similarity between the DNA sequence obtained from the clone pCA759J12-2 and a retina-specific human EST with GenBank accession number AA318352. BLASTX

analysis of EST AA318352 revealed a strong homology of the corresponding protein to a group of *C. elegans* proteins with unknown function (RFP family). The RFP family is known only from *C. elegans* genome and EST sequences (e.g., *C. elegans* C29F4.2 and B0564.3) and is named for the amino acid sequence RFP that is invariant among 15 of the 16 family members; members share a conserved 300-400 amino acid sequence including 25 highly conserved aromatic residues.

A human gene partially represented in pCA759J12-2 and EST AA318352 was dubbed CG1CE (Candidate Gene #1 with the homology to the C. elegans group of genes) and selected for detailed analysis.

BioInformatic Analysis of Assembled DNA Sequences

When the assembled DNA sequences from the nine BMD PACs approached 0.5-1-fold coverage, the DNA contigs were randomly concatenated, and prediction abilities of the program package AceDB were utilized to aid in gene identification.

In addition to the DNA sequence generated from the nine PACs mentioned above, Genbank database entries for PACs 466A11 and 363P2 (GeneBank accession numbers AC003025 and AC003023, respectively) were analyzed with the use of the same AceDB package. PAC clones 466A11 and 363P2 represent parts of the PAC contig across the BMD region (Cooper *et al.*, 1997, Genomics 41:185-192); both clones map to the minimum genetic region containing the BMD gene that was determined by recombination breakpoint analysis in a 12-generation Swedish pedigree (Graff *et al.*, 1997, Hum. Genet. 101: 263-279). Database entries for PACs 466A11 and 363P2 represent unordered DNA pieces generated in Phase 1 High Throughput Genome Sequence Project (HTGS phase 1) by Genome Science and Technology Center, University of Texas Southwestern Medical Center at Dallas.

cDNA sequence and exon/intron organization of the CG1CE gene

Genomic DNA sequences from PACs 466A11 and 759J12 were compared with the CG1CE cDNA sequence from EST AA318352 using the program Crossmatch which allowed for a rapid and sensitive detection of the location of exons. The identification of intron/exon boundaries was then accomplished by manually comparing visualized genomic and cDNA sequences by using the AceDB package. This analysis allowed the identification of exons 8, 9, and 10 that are

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Identification of an additional exon and determination of the exact exon/intron boundaries within the CG1CE gene.

To identify additional exon(s) within the CG1CE gene and verify the exonic composition of this gene, forward and reverse PCR primers from all known
5 exons of the CG1CE gene were synthesized and used to PCR amplify CG1CE cDNA fragments from human retina “Marathon-ready” cDNA (Clontech, Palo Alto, CA). In these RT-PCR experiments forward primer from ex1 (LF: CTAGTCGCCAGACCTTCTGTG) (SEQ.ID.NO.:9) was paired with a reverse primer from ex4 (GR: CTTGTAGACTGCGGTGCTGA) (SEQ.ID.NO.:10), forward
10 primer from ex4 (GF: GAAAGCAAGGACGAGCAAAG) (SEQ.ID.NO.:11) was paired with a reverse primer from ex6 (ER: AATCCAGTCGTAGGCATACAGG) (SEQ.ID.NO.:12), forward primer from ex6 (EF: ACCTTGCGTACTCAGTGTGGA) (SEQ.ID.NO.:13) was paired with a reverse primer from ex8 (AR: TGTCGACAATCCAGTTGGTCT) (SEQ.ID.NO.:14), forward primer from ex8 (AF: CCCTTTGGAGAGGATGATGA) (SEQ.ID.NO.:15) was paired with a reverse
15 primer from ex10 (CR: CTCTGGCATATCCGTCAGGT) (SEQ.ID.NO.:16), forward primer from ex10 (CF: CTTCAAGTCTGCCCCACTGT) (SEQ.ID.NO.:17) was paired with a reverse primer from ex11 (DR: GCATCCCCATTAGGAAGCAG) (SEQ.ID.NO.:18).

20 A 50 µl PCR reaction was performed using the Taq Gold DNA polymerase (Perkin Elmer, Norwalk, CT) in the reaction buffer supplied by the manufacturer with the addition of dNTPs, primers, and approximately 0.5 ng of human retina cDNA. PCR products were electrophoresed on a 2% agarose gel and
25 DNA bands were excised, purified and subjected to sequence analysis with the same primers that were used for PCR amplification. The assembly of the DNA sequence results of these PCR products revealed that:

- (i) exons 1 and 2 from one side and exons 4, 5, 6, 7, 8, 9, 10, and 11 indeed represent fragments of the same gene
- 30 (ii) an additional exon is present between exons 2 and 4 (named ex3)
- (iii) exon 7 (Hypothetical) predicted by the BLASTX analysis is present in the CG1CE cDNA fragment amplified by EF/AR primers.

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Comparison of the DNA sequences obtained from RT-PCR fragments with genomic sequences obtained from pCA759J12-2, PAC 466A11, and shotgun PAC 759J12 subclones was performed using the programs Crossmatch and AceDB. This analysis confirmed the presence of the exons originally found in five ESTs (AA318352, AA307119, AA205892, AA317489, and AA326727) and identified an additional exon (exon3) in the CG1CE gene. Exact sequence of exon/intron boundaries within the CG1CE gene were determined for all of the exons. The splice signals in all introns conform to publish consensus sequences. The CG1CE gene appears to span at least 16 kb of genomic sequence. It contains a total of 11 exons.

10

Two splice donor sites for intron 7 .

Two splicing variants of exon 7 were detected upon sequence analysis of RT-PCR products amplified from human retina cDNA with the primer pair EF/AR. Two variants utilize alternative splice donor sites separated from each other by 203 bp. Both splicing sites conform to the published consensus sequence.

15

Identification of 5' and 3' ends of CG1CE cDNA

RACE is an established protocol for the analysis of cDNA ends. This procedure was performed using the Marathon RACE template from human retina, purchased from Clontech (Palo Alto, CA). cDNA primers KR (CTAAGCGGGCATTAGCCACT) (SEQ.ID.NO.:19) and LR(TGGGGTTCCAGGTGGGTCCGAT) (SEQ.ID.NO.:20) in combination with a cDNA adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC) (SEQ.ID.NO.:21) were used in 5'RACE. cDNA primer DF (GGATGAAGCACATTCCTAACCTGCTTC) (SEQ.ID.NO.:22) in combination with a cDNA adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC) (SEQ.ID.NO.:21) was used in 3'RACE. Products obtained from these PCR amplifications were analyzed on 2% agarose gels. Excised fragments from the gels were purified using Qiagen QIAquick spin columns and sequenced using ABI dye-terminator sequencing kits. The products were analyzed on ABI 377 sequencers according to standard protocols.

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EXAMPLE 2

Best's macular dystrophy is associated with mutations in an evolutionarily conserved region of CG1CE

5 Genomic DNA from BMD patients from two Swedish pedigrees
having Best's macular dystrophy (families S1 and SL76) was amplified by PCR using
the following primer pair:

exG_left AAAGCTGGAGGAGCCGAG (SEQ.ID.NO.:23)

exG_right CTCCACCCATCTTCCGTTC (SEQ.ID.NO.:24)

10 This primer pair amplifies a genomic fragment that is 412 bp long and contains exon4
and adjacent intronic regions.

The patients were:

Family S1:

S1-3, a normal individual, *i.e.*, not having BMD; sister of S1-4

S1-4, an individual heterozygous for BMD; and

15 S1-5, an individual homozygous for BMD.

Patients S1-4 and S1-5 had the clinical symptoms of BMD, including morphological
changes observable upon ophthalmologic examination.

Family SL76:

SL76-3, an individual heterozygous for BMD; mother of SL76-2

20 SL76-2, an individual heterozygous for BMD, son of SL-3.

PCR products produced using the primer sets mentioned above were
amplified in 50 µl reactions consisting of Perkin-Elmer 10 x PCR Buffer, 200 mM
dNTP's, 0.5 ul of Taq Gold (Perkin-Elmer Corp., Foster City, CA), 50 ng of patient
DNA and 0.2 µM of forward and reverse primers. Cycling conditions were as

25 follows:

1. 94°C 10 min

2. 94°C 30 sec

3. 72°C 2 min (decrease this temperature by 1.1°C per cycle)

4. 72°C 2 min

30 5. Go to step 2 15 more times

6. 94°C 30 sec

7. 55°C 2 min

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8. 72°C 2 min
9. Go to step 6 24 more times
10. 72°C 7 min
11. 4°C

5 Products obtained from this PCR amplification were analyzed on 2% agarose gels and excised fragments from the gels were purified using Qiagen QIAquick spin columns and sequenced using ABI dye-terminator sequencing kits. The products were analyzed on ABI 377 sequencers according to standard protocols.

The results are shown in Figure 6. Figure 6 shows a chromatogram from sequencing runs on the PCR fragments from patients S1-3, S1-4, and S1-5. The six readings represent sequencing of both strands of the PCR fragments from the patients. As can be seen from Figure 6, the two patients affected with BMD, patients S1-4 and S1-5, both carry a mutation at position 383 of SEQ.ID.NO.:2. Both copies of the CG1CE gene are mutated in homozygous affected S1-5, while heterozygous affected S1-4 contains both normal and mutated copies of the CG1CE gene. This mutation changes the codon that encodes the amino acid at position 93 of SEQ.ID.NO.:3 from TGG (encoding tryptophan) to TGC (encoding cysteine). Patient S1-3, a normal individual, has the wild-type sequence, TGG, at this codon. This disease mutation that changes this TGG codon to a TGC codon was not found upon sequencing of 50 normal unrelated individuals (100 chromosomes) of North American descent.

Both patients from family SL76 carry a mutation at position 357 of SEQ.ID.NO.:2. This mutation changes the codon that encodes the amino acid at position 85 of SEQ.ID.NO.:3 from TAC (encoding tyrosine) to CAC (encoding histidine). This disease mutation that changes this TAC codon to a CAC codon was not found upon sequencing of 50 normal unrelated individuals (100 chromosomes) of North American descent.

Amino acid positions 85 and 93 of the CG1CE protein are evolutionarily conserved. Figure 7 demonstrates that position 93 is occupied by tryptophan not only in the CG1CE protein, but also in 15 of 16 related *C. elegans* proteins. The lone *C. elegans* protein in which this residue is not tryptophan contains an isofunctional phenylalanine instead. Phenylalanine and tryptophan, both being hydrophobic, aromatic amino acids, are highly similar. Position 85 is occupied by

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tyrosine and isofunctional phenylalanine in all 16 related *C. elegans* proteins. Phenylalanine and tyrosine, both being aromatic amino acids, are highly similar.

EXAMPLE 3

5 Expression of CGICE

RT-PCR: RT-PCR experiments were performed on “quick-clone” human cDNA samples available from Clontech, Palo Alto, CA. cDNA samples from heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and retina were amplified with primers AF (CCCTTTGGAGAGGATGATGA) (SEQ.ID.NO.:15) and
10 CR (CTCTGGCATATCCGTCAGGT) (SEQ.ID.NO.:16) in the following PCR conditions:

1. 94°C 10 min
2. 94°C 30 sec
3. 72°C 2 min (decrease this temperature by 1.1°C per cycle)
- 15 4. 72°C 2 min
5. Go to step 2 15 more times
6. 94°C 30 sec
7. 55°C 2 min
8. 72°C 2 min
- 20 9. Go to step 6 19 more times
10. 72°C 7 min
11. 4°C

The CGICE gene was found to be predominantly expressed in human retina and brain

25 *Northern blot analysis:* Northern blots containing poly(A+)-RNA from different human tissues were purchased from Clontech, Palo Alto, CA. Blot #1 contained human heart, brain placenta, lung, liver, skeletal muscle, kidney, and pancreas poly(A+)-RNA. Blot #2 contained stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, and bone marrow poly(A+)-RNA.

30 Primers CF (CTTCAAGTCTGCCCCACTGT) (SEQ.ID.NO.:17) and exC_right (TAGGCTCAGAGCAAGGGAAG) (SEQ.ID.NO.:25) were used to amplify a PCR product from total genomic DNA. This product was purified on an

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WHAT IS CLAIMED:

1. An isolated DNA comprising nucleotides encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:3, SEQ.ID.NO.:5, and SEQ.ID.NO.:29.
2. The DNA of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ.ID.NO.:1, SEQ.ID.NO.:2, SEQ.ID.NO.:4, SEQ.ID.NO.:28, positions 105-1,859 of SEQ.ID.NO.:2, positions 105-1,409 of SEQ.ID.NO.:4, and positions 11-1,663 of SEQ.ID.NO.:28.
3. An isolated DNA comprising a sequence that is identical to SEQ.ID.NO.:2 except that it contains a different nucleotide at a position selected from the group consisting of positions 120, 121, 122, 357, 358, 359, 381, 382, 383, 783, 784, 785, 999, 1000, and 1001.
4. An isolated DNA that hybridizes under stringent conditions to a nucleotide sequence selected from the group consisting of: SEQ.ID.NO.:1, SEQ.ID.NO.:2, SEQ.ID.NO.:4, and SEQ.ID.NO.:28.
5. An expression vector comprising the DNA of claim 1.
6. A recombinant host cell comprising the DNA of claim 1.
7. A CG1CE protein, substantially free from other proteins, having an amino acid sequence selected from the group consisting of SEQ.ID.NO.: 3, SEQ.ID.NO.:5, and SEQ.ID.NO.: 29.
8. The CG1CE protein of claim 8 containing a single amino acid substitution.
9. The CG1CE protein of claim 9 where the substitution occurs at position 6, 85, 93, 227, or 299.

5 11. The CG1CE protein of claim 8 containing two amino acid
substitutions.

13. An antibody that binds specifically to a CG1CE protein where
the CG1CE protein has the amino acid sequence selected from the group consisting of
15 SEQ.ID.NO.:3 and SEQ.ID.NO.:5.

20 (a) providing a DNA sample from the patient;
(b) providing a set of PCR primers based upon SEQ.ID.NO.:2 or
SEQ.ID.NO.:4;
(c) performing PCR on the DNA sample to produce a PCR
fragment from the patient;
(d) determining the nucleotide sequence of the PCR fragment from
25 the patient;

15. The method of claim 15 where the DNA sample is genomic DNA.

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16. The method of claim 15 where the DNA sample is cDNA.

17. A DNA or RNA oligonucleotide probe comprising at least 18 contiguous nucleotides of at least one of a sequence selected from the group
5 consisting of: SEQ.ID.NO.:1, SEQ.ID.NO.:2, SEQ.ID.NO.:4, and SEQ.ID.NO.:28.

18. A method for determining whether a substance is an activator or an inhibitor of a CG1CE protein or a mutant CG1CE protein comprising:
(a) recombinantly expressing CG1CE protein or mutant CG1CE
10 protein in a host cell;
(b) measuring the biological activity of CG1CE protein or mutant CG1CE protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CG1CE protein or mutant CG1CE protein;
where a change in the biological activity of the CG1CE protein or the
15 mutant CG1CE protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CG1CE protein or mutant CG1CE protein.

TITLE OF THE INVENTION
BEST'S MACULAR DYSTROPHY GENE

ABSTRACT OF THE DISCLOSURE

- 5 Novel human and mouse DNA sequences that encode the gene
CG1CE, which, when mutated, is responsible for Best's macular dystrophy, are
provided. Provided are genomic CG1CE DNA as well as cDNA that encodes the
CG1CE protein. Also provided is CG1CE protein encoded by the novel DNA
sequences. Methods of expressing CG1CE protein in recombinant systems are
10 provided. Also provided are diagnostic methods that detect patients having mutant
CG1CE genes.

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1  ccaaaaaaatt gttctcttgg gggttggggc gacaagcggg aagggagggc
51  attttgggca aattggctta ttgccacgca agggctttaa caccttaggt
101 tgggtgggttc acaggttgca ggcaaccac catggcacac gtatacctat
151 gtaaccaacc tgcaccatca tgtataccta tgtaaccaac ctggtacatt
201 ctgcacacgt atcccaggac tttagagtga aaaaaaaagt ggtgtgtaga
251 aaaatcacct gcaatctcag catagttaac gcttagtaca tttcagagag
301 agagggtgac aggaaaggga ggatgagagt gggtttaaga cacaaggtca
351 tattataaaa tcagggtctc tggaagttta gtcccaaaac cacacatctc
401 ataatcccct gcagtgttgg attaaaatgc aacatcacta aggccacaga
451 ctcagactct ggagaaagat ccagaaaact gcccgtttaa taaacatttg
501 ggcgattctt acggcctcta aagaccaaga accactgctg cctagagctc
551 tgctctcttc attgaacaat acaagaggag tgtgtaggta gacaccacc
601 acttccaaca gcttaggaga gcccttgagt atggattgat gtattaaaat
651 ttattgaatc acatgctgag attttcacca gctgcccgtg gggatctggg
701 catttattcc catattgcac tggctggctg gaagccagca gcataaactc
751 cagggtgtgt ctgtcaacct ccaccagact cacccccctc caccagcccc
801 ggcaggcttc tccttccatc tctctgaagc aacttactga tgggccctgc
851 cagccaatca cagccagaat aacgtatgat gtcaccagca gccaatcaga
901 gctcctcgtc agcatatgca gaattctgtc attttactag ggtgatgaaa
951 ttcccaagca acaccatcct tttcagataa gggcactgag gctgagagag
1001 gagctgaaac ctaccggggg tcaccacaca caggtggoaa ggctgggacc
1051 agaaaccagg actgttgact gcagcccgtt attcattctt tccatagccc
1101 acagggtgtg caaagacccc agggcctagt cagaggctcc tccttctctg
1151 agagtctctg gcacagaagt tgaagctcag cacagcccc taacccccaa
1201 ctctctctgc aaggcctcag gggtcagaac actggtggag cagatcattt
1251 agcctctgga ttttagggcc atggttagag ggggtgttgc ctaaattcca
1301 gccctggtct cagcccaaca ccctccaaga agaaattaga ggggccatgg
1351 ccaggctgtg ctagccgttg cttatgagca gattacaaga agggactaag
1401 acaaggactc ctttgtggag gtccctggctt agggagtcaa gtgacggcgg
1451 ctcagactc acgtgggcag tgccagcctc taagagtggg caggggact
1501 ggccacagag tccCAGGGAG TCCACCAGC CTAGTCGCCA GACCTTCTGT
1551 GGGATCATCG GACCCACCTg gaaccccacc tgtgagtaca aggtgcccc
1601 ggtggactgg gatggggctt tgaggccttc agggttggat ggccatcttg
1651 cgtatttgtg tgggatatgc acacacaggc agcacatgcg caggtgtgtg
1701 ggcacctgtg tgtctgtgca aatgccctga ggtgggaatg agcttgggtg
1751 gcatcaggag cgacagccag ccagtgtggc tgcagcaaaa cacacaggga
1801 aagaatggag ggggcatcaa tcaactgaca aattatttat agagctcccc
1851 ctaaaaaaaaa gaaggtctct tctttcgata gaagaaggga gagaggggtg
1901 ttgtccttat aaatataagg gaggagccgc ccctcaaaaa ataagggagg
1951 gaggacceaa gaccccggtg gttgtgtgtt ttccaggggg agctcgaacc
2001 cttagagggg agcgtgggag aaccgctgta ttcaggcctc tcgagagaaa
2051 aggagcggcc gcccaaaaaa tatccctccc gggcgataag aaatggtggc
2101 ctctctcaaa aagatgaaga ggaagccgga gttgtatgtg ttgatatttt
2151 taaaactcca ggtagnnnnn nnnntgctt cagtaaattt ttattgagcg
2201 ccttctacga gaacacaaga ggagcttcca ttctgaggag gaaacaggca
2251 ggaacaggc agatatcctg tataatttca agtagtgata agtgctctct
2301 agaaatatca agcaaggatg ggagacacag agcaccggtg gcagtggggc
2351 tctatttcca ggttggatgg ttgggaacat cctttctaaa gggaacctgg
2401 agtgggaagg aaccatgcag gtatctcagg aagagcttcc tccaggcagg
2451 aagatcagca ggtggaaagg ccctggagcc accattcagt aaacatcatt
2501 tgagcatctc taccagctag gttccattat gggaatggga atatggtggt
2551 ggacagggct gcctgggtccc ttccatactt ctcacactag ggtgggttag
2601 agagcttggg agctaacgaa caagatgggc tgagaacact gcctagccca
2651 gaggacctga gcttagtgtg tagacattgc tgctgttact gcctttgtcg
2701 ttgtattatt tattttatita tttattgatc ttaagacaga gttttgctct

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FIG.1A

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2751	tcttaccag	gcttgagtg	aatggcgta	tctcagctca	ctgcaacctc
2801	cacctcctgg	gatcaagcga	ttctcctgcc	tcagcctcct	gagtagctgg
2851	gattacaggc	acccgcacca	cgcctggata	atTTTTTgt	atTTTTtagta
2901	gagacaggg	ttcaccatgt	tggacaggct	ggtctcgaac	tcctgacctt
2951	aggtgatcca	cctgcctcga	cttcccaaag	tgatgggatt	ataggcatga
3001	gccactgcgc	ccagtgatta	tagaaagtta	aaggcacatg	gcaatgcaca
3051	cgcttatcta	cgtcttcctc	gccaaagcaa	agggcagcct	ctgggctcac
3101	tttcttgcg	ttctacttcc	aaaaggcagt	cagaactggc	agggccttgg
3151	agaccacttc	atccacctcc	tagggctcct	atgggagagt	tgaggctccag
3201	agcaggggaag	ggctctgaca	ggctctgacc	agggcctctg	atccctacaa
3251	acccccaatc	ggtgtccctc	tctaccagGA	CCCAAGCCCA	CCTGCTGCAG
3301	CCCACTGCCT	GGCCATGACC	ATCACTTACA	CAAGCCAAGT	GGCTAATGCC
3351	CGCTTAGGCT	CCTTCTCCCG	CCTGCTGCTG	TGCTGGCGGG	GCAGCATCTA
3401	CAAGCTGCTA	TATGGCGAGT	TCCTAATCTT	CCTGCTCTGC	TACTACATCA
3451	TCCGCTTTAT	TTATAGgtaa	agctggcagg	gctggggccgg	ggggcctggg
3501	aaggatgtgg	ctggggctgg	gagctgggag	ctcctggggg	ctccccagcc
3551	agctcagggc	ccagtgcacc	agtccactac	aacactaagc	tgggctcctg
3601	accagctcct	gggcactgga	gctgaggctg	cgcgctgggg	gctgggcaga
3651	gtaaagaagt	cacactgaga	ggatgctcaa	gccaggccag	cagggtttta
3701	gccacccttc	ctccaacccc	aggaggaccc	ctggagccca	ggctttgtct
3751	ggccccactc	tactggcctg	ttttactgaa	tcccacacag	actcataggc
3801	ccacatagta	cattaaaaaa	gagagagaga	gagagagaga	gagagagatg
3851	gagtctcact	gtgttgctca	ggctggcttc	gaactcctag	gtcaagcaa
3901	tccccctgcc	ttagcctccc	aaggggctgg	gattacaggt	gtgagctact
3951	gcacttgacc	aaccacatgg	tacttttttt	tttttttttt	ttttttgaga
4001	cagggtttca	ctccatcacc	caggctggag	tgcagtgggg	gcaatcttgg
4051	ctcactgtaa	cctctgcctc	ccagggtgcaa	gcgattctcc	tgccttagcc
4101	tcctgagtag	ctggaattat	aggcacacac	caccacgcct	ggctaatttt
4151	tttttttttc	tgtattttta	gtagagacag	ggtttcatca	tgttgagacg
4201	ctgtgctctg	aaccctgac	ctcaagtgat	ccaccacact	cggcctccca
4251	aagtgtctgg	attacaggtg	tcagccacca	tgcacagccc	acatgggtaca
4301	tttttttaaa	ttatttttta	attaaantgt	ttatctaagg	ccagtagcag
4351	tgactcgcgt	ctgtaatccc	agcactttga	ggggccaagg	tgcggggatc
4401	acttgagcct	gggagttcag	cgtgggcaac	atagttagac	cccgtctcta
4451	ccaaaaattt	aaaaaattag	ctgggagtg	tggcatttgc	ctgtggtccc
4501	agctacttgg	gaagctgagg	tgtggggatg	gctgaagcct	gtgaggtcga
4551	ggctgcagtg	agctatgac	acaccactgc	acttcagcct	agtgacagg
4601	ctatctcaaa	agcaaacaaa	ataatgttta	tctaaacggt	aaggtataat
4651	cacagaatat	atgatagcat	tttaaattga	aaaagcatta	atgattacat
4701	ggattgtaaa	atatcaaata	catgaaattc	ttgtgttctt	aataatgcta
4751	gcaacaaggc	acatttggtt	tttactaggg	caccaaggta	ctttaaaaaa
4801	agttagggcc	agccacaggg	gctcacacct	gtaatcccag	cactttggga
4851	ggccaaggca	ggaggatcac	ttgagcccag	gagtttagga	cctgagcaac
4901	ataggagat	cctgatcttg	tctctataaa	aaattaaaaa	attggctagg
4951	ccctttggct	tacaccgcta	atcccagcac	tttgggaggc	cgaggcgggt
5001	ggatcatgag	gtcaggagtt	caagaccagc	ctggccaaca	tagtgaaccc
5051	aatctctact	ataaatacaa	aaattagccg	agtggggtgg	cacgcacctg
5101	tagttccagc	tactcaggag	gatgaggccg	gagaatcgct	tgagcccggg
5151	aggcagaggc	tgacgtgagc	cgagaccatg	ccattgcact	ccagcctagg
5201	tgacagagt	agactccgtc	ttaaaataat	attaaaatct	taaaatgatc
5251	tgggcatggt	ggcttatgcc	tgtagtccca	cccagctctt	caggaggctg
5301	aagcgggagg	attgtcttcag	cccaggaggt	tgaggctgca	gtgagtcatg
5351	actgtgccgc	tgcccttgag	cctgggtaac	agagcaagac	cctatctcaa
5401	aacaaacaaa	caaacaaaca	aacaaacaaa	aaccaataaa	ccaaaaacat
5451	ttatctaaac	aataaaataa	aggacagata	taatcaccga	atatatgata

FIG. 1B

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5501 gcatttttaaa ttgaaaaagc actaatgact acaatggatt ataaaacatc
5551 aaatacataa aattcttaag ttctctctaa taccaaatac aaagcacatt
5601 ggtcttttgg ttttacttgg gcaccaatgc atgctgaaaa agagtcgttc
5651 attttttaga gtagtttttag gttcacagca aaattgagca gaaggtagag
5701 ttctcatgtg tctctttgct cctcacctcg cccccagcct cccctactatc
5751 aacaccccc cactacagtg gtagatttat tacaatccct gaaccacag
5801 tgacacatca ctatcaccca aagttcatag cgtacagcag gggtcactct
5851 tgggcagtac attccatggg ttgggataaa tgtgtaatga tgtctccacc
5901 atcacagcat caggcagagt agtttcaact ctctaacaaa atcctctgcc
5951 tattcacccc tctcattaaa gccaaacact ctgtttcctt ttttcctttt
6001 agagacagtg tctcgctctg tcaaccaggc tgaagtgcaa tggcaatcac
6051 agcccattgc agcctccaac tcttgggctc aagtgatcct cctatctcag
6101 cctccagtgg ctacgactgc aggcatacgg caacggcacc caactaattt
6151 tttgtagaga tagggctctg ctatgttgac caggatggtc ttgaactctt
6201 ggtcctgcct tagcctccca gagctctggg attacaggcg tgaaccaccg
6251 tgcccgtccc aaacactctg ttctgacctg cttttaaaaa actgacctt
6301 ggatgcattc aaaggatcag ggtgtctgaa actggcctct gcagcaggac
6351 ctctcttctt acacatctcc cagtggccag tgtgaggatt ctccccacaa
6401 gaaaccactg gagggggcct cctcctgtcc gggtttgggg ctgtacaagg
6451 agcatcatgg acctggctca ggctcagga ggggccctgg gctggggaaa
6501 atgtgggata gcatcgaggc agtcccactc ctaccagggg ccgggctaga
6551 ctgtgggaca gtctcagcca tctcctcgct gcgtccacac aattccaccc
6601 ccacccccac ccccagGCTG GCCCTCACGG AAGAAACA GCTGATGTTT
6651 GAGAAACTGA CTCTGTATTG CGACAGCTAC ATCCAGCTCA TCCCCATTTC
6701 CTTCGTGCTG Ggtgagttcc cccttctggc tgttcgggt ccctgtggcc
6751 gcccaggctc cagacaggcc aggggaggat cacgaggagc tgcggcaagg
6801 ggctggggag ggggcggggg aacgccagcg gcaggtcggc gcctctctgt
6851 agggaaaggt gcggactgca gccagagaaa ctgaagttag acgttaggta
6901 acagctcctg ccgttagcaa tgaaaacccc attttctgag ggaagcgctg
6951 acatcatggt ccctggagcc cctgcgcggg aggggagggg gtctggcgga
7001 tttctgggac cagcaggggg accccgggt gacagaaccc ttggggctct
7051 cgcgctcca tgagaggctc tgctgcctc tcgctcccga gcgccttcca
7101 ggagggctgg gggctaggcc cgctcgagc agaaagctgg aggagccgag
7151 gcatcgccgg gcgctgggcc ctgggctctg gccgcagact ggccccctgc
7201 ccctcgcccc ccgccccctc tgcccagGCT TCTACGTGAC GCTGGTCTGT
7251 ACCCGTGGT GGAACCACTG CGAGAACCCTG CCGTGGCCCG ACCCGCTCAT
7301 GAGCCTGGTG TCGGGCTTCG TCGAAGGCCAA GGACGAGCAA GGCCGCTTGC
7351 TGCGGCGCAC GCTCATCCGC TACGCCAACC TGGGCAACGT GCTCATCTGT
7401 CGCAGCGTCA GCACCGCAGT CTACAAGCGC TTCCCCAGCG CCCAGCACCT
7451 GGTGCAAGCA Ggtgggcgga ccgggagcaa cggggaggca ccgggcagag
7501 ccaggggccg agatgggcgc ggcaggaacg gaagatgggt ggagccaaag
7551 tcacccggac tcgggggact gggtggagcc aggagtgggg tgtggtcaag
7601 atttgggggt ccaattgggc gggacagagt cgggtgtctg aaggtggggc
7651 gaggccagga gcccaccctc cgagagttag agtcctgaggc agggataagg
7701 acccttgagg gataatggaa agaagggtga cggcttggga actggtgagg
7751 tactagggtc tacttccctc tgcccttgcc cctcttgatc tccggtttcc
7801 actctggagg tatgggacat tgtctctga cacccccctca gcctggcctg
7851 acctggtcct ggttaataag acagaccag gctaggcgctg gtggctctcg
7901 cctgtaatcc cagtgcctta ggaggcaaa gtgggaagat cgcttgagcc
7951 cagctgtttg agacgcacct gagcaacata gcgagacccc catctctaca
8001 aaaacattaa aaattagcag ggcatgggtg cgtgtgcctg tagcttgagg
8051 ctgagtatcg ggaggctgag gcagaggat cacttgagcc cagcagttcc
8101 aggctgcagt gcgctaagat cgcaccgctg cactccaacc tcggtgacag
8151 agccagaccc tttctctgga aataaataaa taccctgccc acatgctcag
8201 cacagaacag cacctagtag gtgctcagaa atttttttgt tgttgaaaga

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FIG.1C

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8251 aagaggatgg caaaggagtg ctgaggttcc tataggtcag caggtgccgg
 8301 ccatcccttc tgcaggttct cccaccacc gccttcttca ctccactctg
 8351 cagGCTTTAT GACTCCGGCA GAACACAAGC AGTTGGAGAA ACTGAGCCTA
 8401 CCACACAACA TGTTCTGGGT GCCCTGGGTG TGGTTTGCCA ACCTGTCAAT
 8451 GAAGGCGTGG CTTGGAGGTC GAATCCGGGA CCCTATCCTG CTCCAGAGCC
 8501 TGCTGAACgt gagcccactg tacagacagg gctgccgcag agtgggaagg
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 8651 gcaatccaca gaccgaggtg gtcccttctc agaggccccct cctcttctc
 8701 caagtctgtg aggtcctggt tcccttttga tagatgagga agctgagaca
 8751 caaagagggt tagtgagctt cccatggcca cacagccagg aatggaccat
 8801 aggtaccagg ccctggtacc tggagaagag gtgggggcga gccaggggtg
 8851 ggggcaggtg gtgttcagaa ccccatcccc ctcttctgcc cccagGAGA
 8901 TGAACACCTT GCGTACTCAG TGTGGACACC TGTATGCCTA CGACTGGATT
 8951 AGTATCCAC TGGTGATAC ACAGgtgagg actaggctgg tgaggctgcc
 9001 cttttgggaa actgaggcta gaaggaccaa ggaagcagct ggggtgggaa
 9051 gggctcacct agaggctaag tggctcccct gggagtggg tccacacttt
 9101 gaagttgggt ctggactttg aagtgccaa ttctaagagt ccaggctcct
 9151 gcctggccca gtccagtaga ggcaatgtga ttatccccat attaaagaga
 9201 ggttggcagg gcacagtggc tcatgcatgt aatcccagca ctttgggaag
 9251 ctgaggcagg tggatcacct gaggtcagga gttcgagacc agcctggcca
 9301 acatggtgaa accccatctc tactgaaaat acagaattag ctgtgtggtg
 9351 gtgcacgcct gtaatcacag ctacttgga ggctgaggca ggagaatagc
 9401 ttgaaccggg gaggtggagg ttgcagttag ctgagatcat gccactgcac
 9451 tccagcctgg gcgacacagc aagactctgt ctcaaacaaa caaacaacaa
 9501 aacaacaaaa caaacaacaa aaggggttaa cagagcccct aagtcacata
 9551 agtgtgcaag tcagaacaag gccttgggtc cctgtctcag actcccagcc
 9601 cctggagcat cctgatttca gggttcccac cttagcccttt gctaccacat
 9651 cctcctctc ctcctctctc tcccagGTGG TGA CTGTGGC GGTGTACAGC
 9701 TTCTTCTGA CTTGTCTAGT TGGGCGGCAG TTTCTGAACC CAGCCAAGGC
 9751 CTACCCCTGGC CATGAGCTGG ACCTCGTTGT GCCCGTCTC ACGTTCCTGC
 9801 AGTTCTTCTT CTATGTTGGC TGGCTGAAGG TGGGCTCTC CAGGGCCCTG
 9851 CTGGGCTGGA GGCATGGCCA GAGGGGTCAT GGCCAGCAGC TGCTTGAGAC
 9901 CAGGATGCAG TGTAGGAAA GGAAGGCTC ACGGGTAGAA AGCAGCCAGG
 9951 CGTGGTGGCG CACACCTGTA ATCCCAGCTA CTCGGGAGGC TGAGGCAGGA
 10001 GAATCGCTTG AACCCGGGAG GCGGAGGTTG TGgtgagttg agatcgtgcc
 10051 actgcactcc agcctgggca aaagaatgaa actctatctc aaaaacaaca
 10101 acaacaacaa aacaaagccc taagggtcag aagcccctgc cctttagaag
 10151 cagagcgaac actctcctat taagatgctg ttgggtgtct ttttactca
 10201 gtagctgtcc agtattctcc acacagcata atagacagat tctaatacaa
 10251 atttcttcaa ctcttaattc ctccctttgt ccaccatttt ttcttctacc
 10301 tcctaattta tgaatgggtt agtatgctct gcttctgcat tgagacaaaa
 10351 tacagagaga gagaaagatc tatcttaatc ccgccccatt ttagttggaa
 10401 aaaaacttta ttaaatcagg caagtaaaat ccgccaagga ttgnnnnnnn
 10451 nnnagatggt ctgaatcaga gaggtttctc tcgagctctt tatctttcct
 10501 tccttatggt gccaccacac tctctctcac ttctacctt cctttatatt
 10551 ttggtaatgg ggggtgaagt ctctgtctct gcccttctg tcaactgtgac
 10601 acacacacac acacacacac acacacacac acacacacac attcatattc
 10651 ctctaaattc cccctgcacc cccagttatc ttgggtttct gcagatcaaa
 10701 acaaatcaca cttttatgct tgaaattctc cagggtgccc cagtggcctg
 10751 caagatgtcc cctggacccc taaggcagac gcgtgtcacc tcttcggggc
 10801 tttgttaggg catttttagag gttgctatcc aggaatctgc ccacctagac
 10851 tgccctttag ttcagccagc cttcagtata tatctctggt gcatgaatga

FIG.1D

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10901	ataaaattat	gcaactccag	gtaagataca	tgaggtgaga	taaaggcagt
10951	gactcagccg	agtgatacac	tcagggaacag	ctglgggtgl	tcagggaagg
11001	actggctcag	aagagttaga	ggggctgtgt	ccagaagtgt	gtgggtgcct
11051	acaagtgtgg	ggggctggag	ccctaaactc	tgcctttgaa	gacagtggtc
11101	aggcaggaag	ggcttcatgg	ggtgtggaaa	tagcagcagc	tgaggtttaa
11151	agggggaagc	tggctttgag	gagttctgcc	tgagggttta	cagagcctca
11201	cctgtcccca	agGTGGCAGA	GCAGCTCATC	AACCCCTTTG	GAGAGGATGA
11251	TGATGATTTT	GAGACCAACT	GGATTGTCGA	CAGGAATTIG	CAGgtatggg
11301	gagagggaga	gaaaccatac	catggacctt	cccaaagtgt	gacccaaaga
11351	gagctcctcc	ctcctgcagc	cagtcattca	ctcacaggat	tctcacctca
11401	atctttgagg	ctgcaggcag	gcacccatct	ccccatttca	caggcagggg
11451	aactgaggtc	cagagagagg	gagagatCcc	tccaagtcat	caggcacata
11501	caaggtcctg	cctgggatga	tctttctgtg	ggacttcttc	tgtccctggg
11551	gaccagGTGT	CCCTGTTGGC	TGTGGATGAG	ATGCCACCAGG	ACCTGCCTCG
11601	GATGGAGCCG	GACATGTACT	GGAATAAGCC	CGAGCCACAG	CCCCCTACA
11651	CAGCTGCTTC	CGCCAGTTC	CGTCGAGCCT	CCTTTATGGG	CTCCACCTTC
11701	AACATCAGgt	gtggccagag	ccaggggggt	gggtgggaag	ccctcctag
11751	tgcaggggtc	tgcctaggaa	cttagaatag	cactagttaa	tgcatacagg
11801	ttgcttcagt	aagtgtcagg	cactgtacta	tgtcttttat	aaacattaac
11851	tatttttttc	ctccaataaa	ttctggtttg	ttatcccaag	ttthcagata
11901	attaaagtac	aggttcagag	agagtaagtt	gtccaaggcc	acatagctac
11951	caaagtgtgc	atttgctact	cgaaggacag	cctatgatca	gtgatgcagt
12001	ggaacgttag	gacctggctc	ttgtcatcca	gaactatgtt	ttcttttctt
12051	tttgagacag	tatctcgctc	tgtcgcccag	gttgagcgc	agtggcgtga
12101	tcttggtcca	ctgcaacctc	cgctcctgg	gttcaagtga	ttctcctgct
12151	tcagcctccc	cagtagctgg	gattacaggt	gcccacaacc	acaactggct
12201	aatttttgta	cttttagtag	agatgaggtt	tcaccatgtt	ggccaggctg
12251	gtctccaact	cctgaccagt	aatctgcccg	ctttggcctc	ccaaaatgct
12301	ggaattatag	gtgtcaaaac	tatgttttct	gataagctac	gatgcttgga
12351	tgggaagtgg	aagtggggtt	ccctgggatg	ggggaggggc	agcaaagtcc
12401	cagcaggcag	ccaggccatc	acaggtacct	cctgaattga	ctttgtccta
12451	ccgagtaaag	ggctcaggcc	accacacagca	gccagactta	tccccactag
12501	gtcccacttc	cctgattcca	tctgaatccc	tcttgagctg	cagtgggctg
12551	aagggtatc	ccagctggtc	ctttctcccc	aggacaacag	agttgaaagt
12601	gccttgagga	gtgttgggca	catgtcaggg	ttcatactca	agggtttctt
12651	ccacggatc	cagtgtgtgt	ctcgcttgtt	cttttctttt	ttttttttta
12701	aacggagttt	cactcttggt	gcccagagct	ggagtgcagt	ggcataatct
12751	cggtcactg	caacctccgc	ctcccagatt	caagcaattc	tcctgcctca
12801	gcatacctgag	tagctgggat	tataggtgcc	agccaccaag	cccggctaata
12851	ttttgtattt	ttagtagaga	cagtttcacc	atgttggcca	ggctgggtctc
12901	gaactcctga	cctcaggtga	tccaccctcc	tcagcctccc	aaagtgtctg
12951	gattacatgt	gtgagccact	gtgcctggct	gcttgttctt	ttaagaacca
13001	aatatcctac	tagactgcaa	tcgagtttaa	ctacagtcta	tagatactgt
13051	gaggaatggt	tgggaagggtc	atcaaatgaa	ggctggaggc	ttgcttaggt
13101	cagaaacatt	tctggaggat	gactttgagc	cctaactggt	ctgtacccca
13151	gcagctgaag	gttgttgagg	gatggggagg	gctgaaaaca	gaacgataaa
13201	gcatagacct	tgtctccaag	gaatgcacaa	tttatggagg	gagctcaaac
13251	ccaagtctca	aactctggat	acaagggtaca	aagtactgga	tgtccagaaa
13301	agggacagaa	catggaacac	agtcataatt	gtctgcatgg	gaggcggtt
13351	ccagctgggt	ctggagctga	gcatggaac	atgggaagaa	tctgaacttg
13401	ggcaagggca	ggccatactc	tctggtagat	aagctttcct	tgcagggtaa
13451	aggtctgggg	ctcccgggat	gcctgttgct	aggaagtcaa	atttcicctt
13501	gtggatgtca	ctcccagttg	gaaccacaaa	ttcctggcat	tgcccagagt
13551	cactcatggg	cctcatctga	accactcatg	ccagggcacc	agtgtttctg

FIG.1E

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13601 actgcctgga gtgaggggtt ttacagggga agtgaatgat gaggaggcct
 13651 ttacacgccca ggaggggttg ttgcgggggt tggatgttaa ctctggtcaa
 13701 gagggaatca acaaacagtg aggtgagctg ggcctggagg gatcaccggg
 13751 aggtacagta cagatcagga gagaggtgag agctggggca tggtagggaa
 13801 gacggtgtgg ccttggcttg ggccaactga gagagaggag cgggggtaag
 13851 ggagaagtaa ggccaggtgt tggctctttg tccactggct cagccctgca
 13901 tctcctgttt ctttccagCC TGAACAAAGA GGAGATGGAG TTCCAGCCCA
 13951 ATCAGGAGGA CGAGGAGGAT GCTCACGCTG GCATCATTGG CCGCTTCCTA
 14001 GGCCCTGCAGT CCCATGATCA CCATCCTCCC AGGGCAAACCT CAAGGACCAA
 14051 ACTACTGTGG CCCAAGAGGG AATCCCTTCT CCACGAGGGC CTGCCAAAAA
 14101 ACCACAAGGC AGCCAAACAG AACGTTAGGG GCCAGGAAGA CAACAAGGCC
 14151 TGGAAAGCTTA AGGCTGTGGA CGCCTTCAAG TCTGCCCCAC TGTATCAGAG
 14201 GCCAGGCTAC TACAGTGCCC CACAGACGCC CCTCAGCCCC ACTCCCATGT
 14251 TCTTCCCCCT AGAACCATCA GCGCCGTCAA AGCTTCACAG TGTACAGGC
 14301 ATAGACACCA AAGACAAAAG CTTAAAGACT GTGAGTTCCTG GGGCCAAGAA
 14351 AAGTTTTGAA TTGCTCTCAG AGAGCGATGG GGCCTTGATG GAGCACCCAG
 14401 AAGTATCTCA AGTGAGGAGG AAAACTGTGG AGTTTAACCT GACGGATATG
 14451 CCAGAGATCC CCGAAAATCA CCTCAAAGAA CCTTTGGAAC AATCACC AAC
 14501 CAACATACAC ACTACACTCA AAGATCACAT GGATCCTTAT TGGGCCTTGG
 14551 AAAACAGgtc tgtcctccac ctgaaccagg ggcactgcat tgcctgtgtc
 14601 cccaccccag cttcccttgc tctgagccta cccttctctc acaatttctt
 14651 agggttccat cactgccaga gcacactgga cctacgcccc gcaactggctt
 14701 ggggtatata cttggccacc ttcacagggg tccatagggg gtgttcggga
 14751 cctttttctca cttcacccctg gtatcacccg gaagacttct tgggaccagg
 14801 tgaaggaaga tgaggttgtg ctgaccagaa tgctgctgga gaactgcccc
 14851 agggctgaca ggccaggctt agctgagcag atgttatcac tggccccaac
 14901 ttactttgag caaggggtggc tgacccaaaa ccatgaggtg gcagtcagct
 14951 ggatgacaga tgaacacttc cccataact atttagggta gtacacaagc
 15001 actacaggaa aggggtggcag gaactgcctc actcctagga actggtagat
 15051 ggtgaggttg aggggtgtcca gcgccattag gtcattttct cactgccttg
 15101 gaacctcacc aaaatacttc ttgcttcctt ggggtcagcc caaagctgtc
 15151 acaaaatcag atatttccct ttattccaga tttcctggac actgtcaccc
 15201 aattataaac accccacttc agacccaatc acgtgggagg aagtgttaact
 15251 tcccttttct ggattctcaa gcagttactt tcacgggtca gaacacgcag
 15301 ctattatgat tgaaacctta aaagggcaac aatttcaatc ttgcttctag
 15351 gctaagacag gaacttggca aacatctgtg gcctgttcag caaaggatgt
 15401 tcatatttaa gaatcttgtc ttgggctggg tgtggaggca agtgaatcac
 15451 aggaggtcag gagtttgaga ccaacctggc caacatgatg aaaccccatc
 15501 tctaccaaaa aaaatacaaa tcagctggcc gtcgtggtgt gcctgtagtc
 15551 ccaacgcagg aggttgaggg gagaattgct tgaaccagg aggtggtggt
 15601 tgcagtgaga ttgagcaact gcaatccagc ctgggcgacg gagtgagact
 15651 gtctcaaaaa aaaaaaaaaa aggatcgtct caacctttgc cctcctactg
 15701 caacattttg gtatttgaaa tgaaggtacc ttccatactt atgctgttaa
 15751 tactttcatt ctcactagGG ATGAAGCACA TTCC7AACCT GCTTCCTAAT
 15801 GGGGATGCTT CGCCAGCCAG GTCCTCACCT GTGTGTACAC CAGCAGGACA
 15851 CTGATCCAGT CACAGCCATA CAGCTGTCCA CACTGAAGAA CGTGTCCCTAC
 15901 AACAGCCTGA ATCAAATGGT TAGCTTAATA GATAAAAATC CCAGACTACT
 15951 TCAGCCTTTA ATGCCTTTTA TTCATAAAAA CTGTGAAAGC TAGACTGAAC
 16001 CATTGGAAAC ATTTAACTCA GACTCTGGAT TCAGAGTCGG GAACCCCTAG
 16051 TTCTATCTGA ATCCAAGACA GCCACACCTT AGTATACTGC CCAAACCTAAT
 16101 GAGTTTAAATA AATACAAATA CTCGT (SEQ.ID.NO.:1)

FIG.1F

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CAGGGAGTCCCACCAGCCTAGTCGCCAGACCTTCTGTGGGATCATCGGAC 50
CCACCTGGAACCCACCTGACCCAAGCCCACCTGCTGCAGCCCACTGCCT 100
GGCCATGACCATCACTTACACAAGCCAAGTGGCTAATGCCCGCTTAGGCT 150
CCTTCTCCCGCCTGCTGCTGTGCTGGCGGGGCAGCATCTACAAGCTGCTA 200
TATGGCGAGTTCTTAATCTTCTGCTCTGCTACTACATCATCCGCTTTAT 250
TTATAGGCTGGCCCTCACGGAAGAACAACAGCTGATGTTTGAGAACTGA 300
CTCTGTATTGCGACAGCTACATCCAGCTCATCCCCATTTCTTCGTGCTG 350
GGCTTCTACGTGACGCTGGTCTGACCCGCTGGTGGAACCAAGTACGAGAA 400
CCTGCCGTGGCCCGACCGCCTCATGAGCCTGGTGTGGGCTTCGTCTGAAG 450
GCAAGGACGAGCAAGGCCGGCTGCTGCGGCGCACGCTCATCCGCTACGCC 500
AACCTGGGCAACGTGCTCATCCTGCGCAGCGTCAGCACCGCAGTCTACAA 550
GCGCTTCCCCAGCGCCCAGCACCTGGTGCAAGCAGGCTTTATGACTCCGG 600
CAGAACAACAAGCAGTTGGAGAACTGAGCCTACCACACAACATGTTCTGG 650
GTGCCCTGGGTGTGGTTTGCCAACCTGTCAATGAAGGCGTGGCTTGGAGG 700
TCGAATCCGGGACCTATCCTGCTCCAGAGCCTGCTGAACGAGATGAACA 750
CCTTGCGTACTCAGTGTGGACACCTGTATGCCTACGACTGGATTAGTATC 800
CCACTGGTGTATACACAGGTGGTGAAGTGTGGCGGTGTACAGCTTCTTCT 850
GACTTGTCTAGTTGGGCGGCAGTTTCTGAACCCAGCCAAGGCCTACCCTG 900
GCCATGAGCTGGACCTCGTTGTGCCGTCTTCACGTTCTTCAGTTCCTTC 950
TTCTATGTTGGCTGGCTGAAGGTGGCAGAGCAGCTCATCAACCCCTTTGG 1000
AGAGGATGATGATGATTTTGGAGACCAACTGGATTGTCGACAGGAATTTGC 1050
AGGTGTCCCTGTTGGCTGTGGATGAGATGCACCAGGACCTGCCTCGGATG 1100
GAGCCGGACATGTACTGGAATAAGCCCGAGCCACAGCCCCCTACACAGC 1150
TGCTTCCGCCAGTTCGGTCGAGCCTCCTTTATGGGCTCCACCTTCAACA 1200
TCAGCCTGAACAAAGAGGAGATGGAGTTCCAGCCCAATCAGGAGGACGAG 1250
GAGGATGCTCACGCTGGCATCATTGGCCGCTTCCTAGGCCTGCAGTCCCA 1300
TGATCACCATCCTCCCAGGGCAAACCTCAAGGACCAAACCTACTGTGGCCCA 1350
AGAGGGAATCCCTTCTCCACGAGGGCCTGCCCCAAAACCAAGGCAGCC 1400
AAACAGAACGTTAGGGGCCAGGAAGACAACAAGGCCTGGAAGCTTAAGGC 1450
TGTGGACGCCTTCAAGTCTGGCCCACTGTATCAGAGGCCAGGCTACTACA 1500
GTGCCCCACAGACGCCCTCAGCCCCACTCCCATGTTCTTCCCCCTAGAA 1550
CCATCAGCGCCGTCAAAGCTTCACAGTGTACAGGCATAGACACCAAAGA 1600
CAAAAGCTTAAAGACTGTGAGTTCTGGGGCCAAGAAAAGTTTTGAATTGC 1650
TCTCAGAGAGCGATGGGGCCTTGATGGAGCACCCAGAAGTATCTCAAGTG 1700
AGGAGGAAAACCTGTGGAGTTTAACCTGACGGATATGCCAGAGATCCCCGA 1750
AAATCACCTCAAAGAACCTTTGGAACAATCACCAACCAACATACACACTA 1800
CACTCAAAGATCACATGGATCCTTATTGGGCCCTTGGAACACAGGGATGAA 1850
GCACATTCCCTAACCTGCTTCCTAATGGGGATGCTTCGCCAGCCAGGTCCT 1900
CACCTGTGTGTACACCAGCAGGACACTGATCCAGTCACAGCCATACAGCT 1950
GTCCACACTGAAGAACGTGTCTTACAACAGCCTGAATCAAATGGTTAGCT 2000
TAATAGATAAAAAATCCCAGACTACTTCAGCCTTTAATGCCTTTTATTCAT 2050
AAAAACTGTGAAAGCTAGACTGAACCATTGGAACATTTAACTCAGACTC 2100
TGGATTCAAGTTCGGGAACCTTAGTTCTATCTGAATCCAAGACAGCCAC 2150
ACCTTAGTATACTGCCCAAACCTAATGAGTTTAATAAAATACAAATACTCGT 2200
TAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ. ID. NO. : 2)

FIG.2

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MTITYTSQVANARLGSFSRLLLCWRGSIYKLLYGEFLIFLLCYYIIRFIY 50
RLALTEEQQLMFEKLTLYCDSYIQLIPISFVLGFYVTLVVTRWWNQYENL 100
PWPDRMLSLVSGFVEGKDEQSRLLRRTLIRYANLGNVILRSVSTAVYKR 150
FPSAQHLVQAGFMTPAEHKQLEKLSLPHNMFVWPVWFANLSMKAWLGGR 200
IRDPIILLQSLLENMNTLRTQCGHLYAYDWISIPLVYTQVVTAVYSFFLT 250
CLVGRQFLNPAKAYPGHELDLVVPVFTFLQLFLYVGWLKVAEQLINPFGE 300
DDDDFETNWIVDRNLQVSLLAVDEMHQDLPRMEPDMYWNKPEPQPPYTAA 350
SAQFRRASFMGSTFNISLNKEEMEFQPNQEDEEDAHAGIIGRFLGLQSHD 400
HHPPRANSRTKLLWPKRESLLHEGLPKNHKAAKQNVRGQEDNKAWKLKAV 450
DAFKSGPLYQRPGYYSAPQTPLSPTPMFFPLEPSAPSKLHSVTGIDTKDK 500
SLKTVSSGAKKSFELLSESDGALMEHPEVSQVRRKTVEFNLDMPEIPEN 550
HLKEPLEQSPTNIHTTLKDHPYWALENRDEAHS (SEQ. ID. NO.: 3)

FIG.3

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CAGGGAGTCCCACCAGCCTAGTCGCCAGACCTTCTGTGGGATCATCGGAC 50
 CCACCTGGAACCCACCTGACCCAAGCCCACCTGCTGCAGCCCACTGCCT 100
 GGCCATGACCATCACTTACACAAGCCAAGTGGCTAATGCCCGCTTAGGCT 150
 CTTTCTCCCGCCTGCTGCTGTGCTGGCGGGGAGCATCTACAAGCTGCTA 200
 TATGGCGAGTTCTTAATCTTCTGCTGCTGCTACTACATCATCCGCTTTAT 250
 TTATAGGCTGGCCCTCACGGAAGAACAACAGCTGATGTTTGAGAAACTGA 300
 CTCTGTATTGCGACAGCTACATCCAGCTCATCCCCATTTCTTCGTGCTG 350
 GGCTTCTACGTGACGCTGGTCTGACCCGCTGGTGGAAACCAGTACGAGAA 400
 CCTGCCGTGGCCCGACCGCCTCATGAGCCTGGTGTGCGGGCTTCGTGGAAG 450
 GCAAGGACGAGCAAGGCCGGCTGCTGCGGCGCACGCTCATCCGCTACGCC 500
 AACCTGGGCAACGTGCTCATCCTGCGCAGCGTCAGCACCGCAGTCTACAA 550
 GCGCTTCCCCAGCGCCAGCACCTGGTGCAAGCAGGCTTTATGACTCCGG 600
 CAGAACACAAGCAGTTGGAGAACTGAGCCTACCACACAACATGTTCTGG 650
 GTGCCCTGGGTGTGGTTTGCCAACCTGTCAATGAAGGCGTGGCTTGGAGG 700
 TCGAATCCGGGACCCTATCCTGCTCCAGAGCCTGCTGAACGAGATGAACA 750
 CTTGCGTACTCAGTGTGGACACCTGTATGCCTACGACTGGATTAGTATC 800
 CCACTGGTGTATACACAGGTGGTACTGTGGCGGTGTACAGCTTCTTCCT 850
 GACTTGTCTAGTTGGGCGGCAGTTTCTGAACCCAGCCAAGGCCTACCCTG 900
 GCCATGAGCTGGACCTCGTTGTGCCCGTCTTACGTTCTTCAGTTCTTC 950
 TTCTATGTTGGCTGGCTGAAGGTGGGCCTCTCCAGGGCCCTGCTGGGCTG 1000
 GAGGCATGGCCAGAGGGGTCTAGGCCAGCAGCTGCTTGAGACGAGGATGC 1050
 AGTGTGAGGAAAGGAAGGTCTACGGGTAGAAAGCAGCCAGGCGTGGTGG 1100
 CGCACACCTGTAATCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCGCT 1150
 TGAACCCGGGAGGCGGAGGTTGTGGTGGCAGAGCAGCTCATCAACCCCTT 1200
 TGGAGAGGATGATGATGATTTTGAGACCAACTGGATTGTGACAGGAATT 1250
 TGCAGGTGTCCCTGTTGGCTGTGGATGAGATGCACCAGGACCTGCCTCGG 1300
 ATGGAGCCGGACATGTACTGGAATAAGCCCCGAGCCACAGCCCCCCTACAC 1350
 AGCTGCTTCCGCCCAGTTCCGTCGAGCCTCCTTTATGGGCTCCACCTTCA 1400
 ACATCAGCCTGAACAAAGAGGAGATGGAGTTCCAGCCCAATCAGGAGGAC 1450
 GAGGAGGATGCTCAGCTGGCATCATTGGCCGCTTCTTAGGCCTGCAGTC 1500
 CCATGATCACCATCCTCCAGGGGCAAACTCAAGGACCAAACTACTGTGGC 1550
 CCAAGAGGGAATCCCTTCTCCACGAGGGCCTGCCAAAAACCACAAGGCA 1600
 GCCAAACAGAACGTTAGGGGCCAGGAAGACAACAAGGCCTGGAAGCTTAA 1650
 GGCTGTGGACGCCTTCAAGTCTGGCCCACTGTATCAGAGGCCAGGCTACT 1700
 ACAGTGCCCCACAGACGCCCCCTCAGCCCCACTCCCATGTTCTTCCCCCTA 1750
 GAACCATCAGCGCCGTCAAAGCTTACAGTGTACAGGCATAGACACCAA 1800
 AGACAAAAGCTTAAAGACTGTGAGTTCTGGGGCCAAGAAAAGTTTGAAT 1850
 TGCTCTCAGAGAGCGATGGGGCCTTGATGGAGCACCCAGAAGTATCTCAA 1900
 GTGAGGAGGAAAACTGTGGAGTTTAACTGACGGATATGCCAGAGATCCC 1950
 CGAAAATCACCTCAAAGAACCTTTGGAACAATCACCAACCAACATACACA 2000
 CTACACTCAAAGATCACATGGATCCTTATTGGGCCTTGGAAAAACAGGGAT 2050
 GAAGCACATTCTAACCTGCTTCTTAATGGGGATGCTTCGCCAGCCAGGT 2100
 CCTCACCTGTGTGTACACCAGCAGGACACTGATCCAGTCACAGCCATACA 2150
 GCTGTCCACACTGAAGAACGTGTCCTACAACAGCCTGAATCAAATGGTTA 2200
 GCTTAATAGATAAAAAATCCAGACTACTTCAGCCTTTAATGCCTTTTATT 2250
 CATAAAAACTGTGAAAGCTAGACTGAACCATTGGAAACATTTAACTCAGA 2300
 CTCTGGATTGAGAGTCGGGAACCCCTAGTTCTATCTGAATCCAAGACAGC 2350
 CACACCTTAGTATACTGCCCAAACTAATGAGTTTAATAAATACAAATACT 2400
 CGTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA(SAQ. ID. NO. : 4)

FIG.4

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MTITYTSQVANARLGSFSRLLLCWRGSIYKLLYGEFLIFLLCYYIIRFIY 50
RLALTEEQQLMFEKLTLYCDSYIQLIPISFVLGFYVTLVWTRWWNQYENL 100
PWPDRMLSLVSGFVEGKDEQGRLLRRTLIRYANLGNVLILRSVSTAVYKR 150
FPSAQHLVQAGFMPAEHKQLEKLSLPHNMFVWPWWFANLSMKAWLGGR 200
IRDPIILLQSLLNEMNTLRTQCGHLYAYDWISIPLVYTQVVTAVYSFFLT 250
CLVGRQFLNPAKAYPGHELDLVVPVFTFLQFFFYVGWLKVGLSRALLGWR 300
HGQRGHGQQLLETRMQCQERKVS RVESQAWWRTPVIPATREAEAGESLE 350
PGRRLWWQSSSSTPLERMMILRPTGLSTGICRCPCWLWMRCTRCLGW 400
SRTCTGISPSHSPPTQLLPSSVEPPLWAPPSTSA (SEQ.ID.NO.:5)

FIG.5

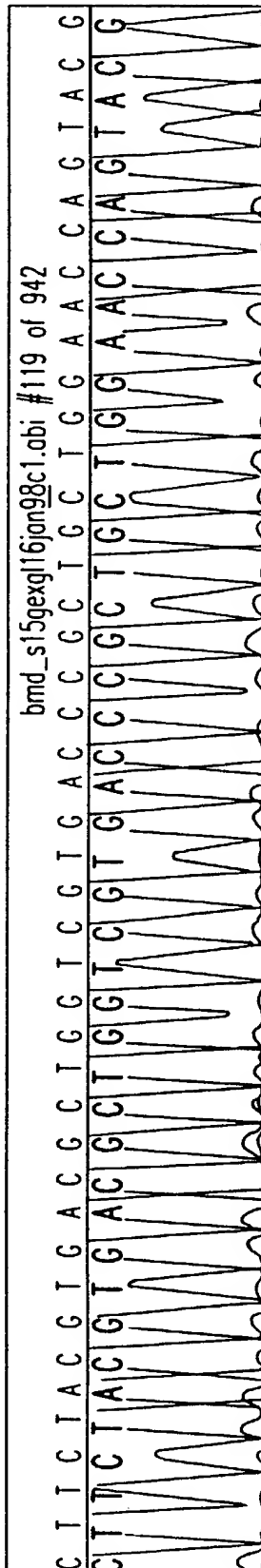


FIG. 6A

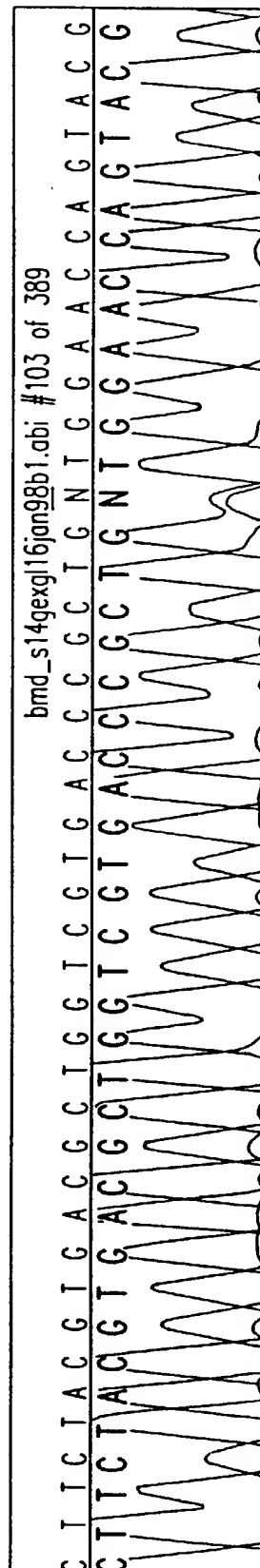


FIG. 6B

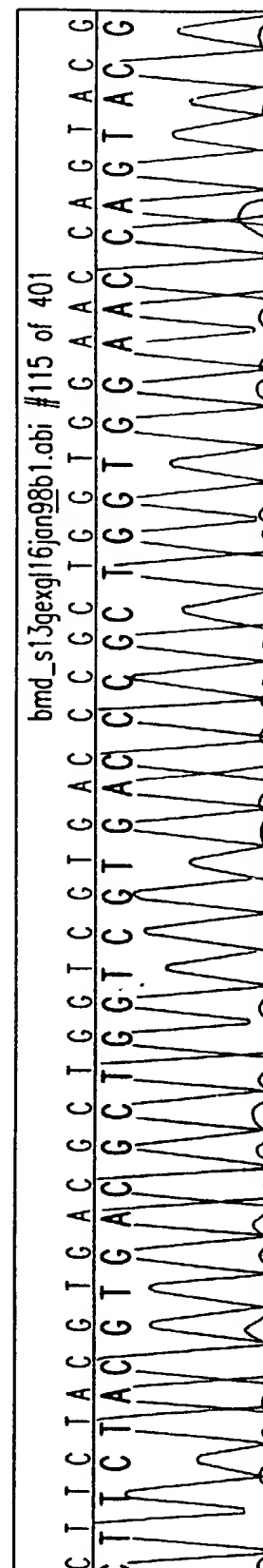


FIG. 6C

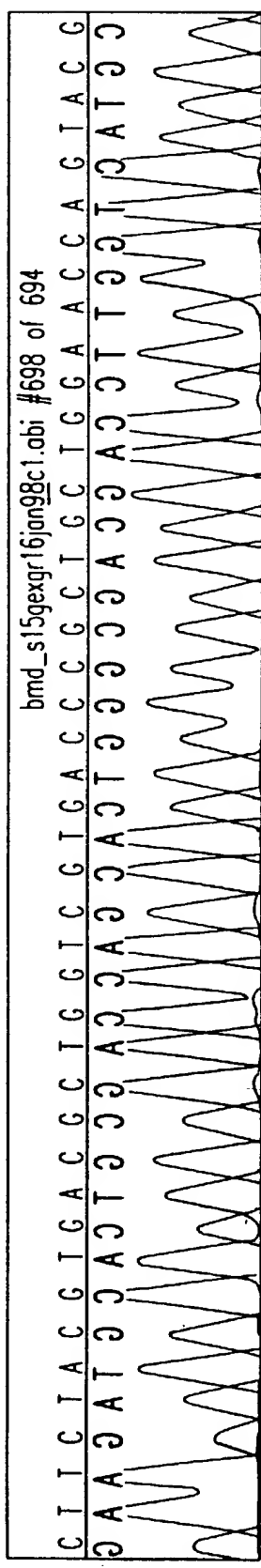


FIG. 6D

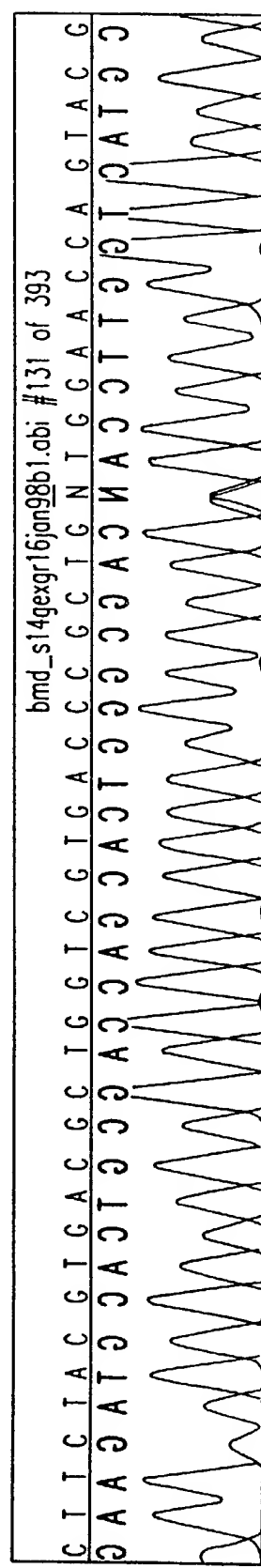


FIG. 6E

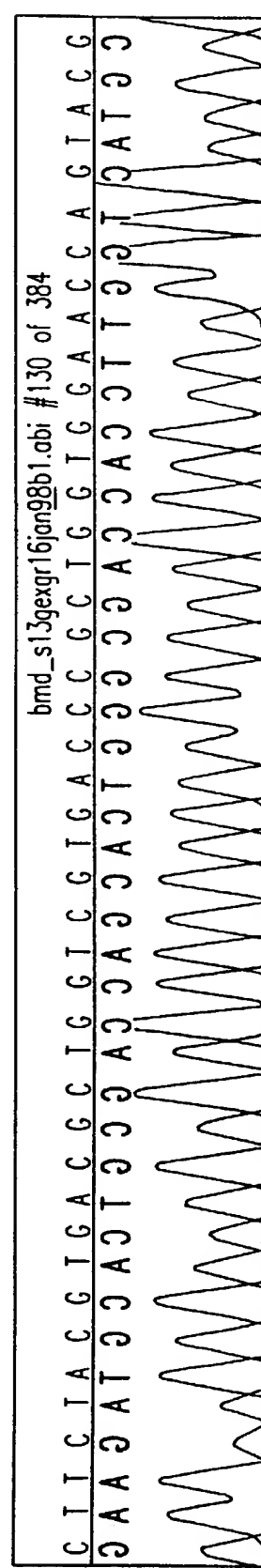


FIG. 6F

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<u>GenBank/SwissProt accession numbers</u>	<u>Protein sequence</u>	<u>SEQ.ID.NO.</u>
CG1CE_protein	IPISFVLGFY VTLVVTRWVN QYENLPWPDR 2 (part)	
af016687 (PID:g2315833)	IPLTFMLGFE VTIIVGRWND IFLNIGWVDN	28
z73105 (PID:e242363)	IPLTFMLGFE VTIIVRRWND IFANLGWVEN	29
z73422 (PID:e244423)	IPLEFVLGFE VTIVVDRWTK LWRTVGFIDD	30
z73422 (PID:e244542)	IPLEFVLGFE VTTVVNRWTK LYQTIGFIDN	31
p34577	VPLDWMLGFE IAGVLRREWY LYDIIGFIDN	32
p34672	IPLNFMLGFE VTAVVNRWTY LYQIIGFIDN	33
p34319	LPLNFVLGFE CNIIIRRWLK LYTSLGNIDN	34
z68335 (PID:e217363)	IPINFMLGFE VTTVINRWMT QFANLGMIDN	35
z68753 (PID:e218704)	IPLTFLLGFE VSFVVARWGS ILNGIGWIDD	36
af025458 (PID:e2429439)	IPVTFMLGFY VSIVYNRWTK VFDNVGWIDT	37
u28412 (PID:g849242)	LPLTFMLGFE VTTVFERWRS ALNVMPFIES	38
u70848 (PID:g1572760)	IPLTFLLGFY VSNVVSRRWR QFETLRWPED	39
z81074 (PID:e351507)	IPLTFLLGFY VSNVVARWWR QFETLYWPED	40
q09379	IPLTFLLGFY VAMIVRRWWD CCQLISWPDH	41
z72509 (PID:e239377)	IPLSFLLGFE VSLIVARWWE QFNCISWPK	42
z83221 (PID:e349023)	VPMQPMLGYE IGMVGERWGE SFENVSYIEK	43

FIG.7

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1 GTGCCAAGCCATGACTATCACCTACACAAACAAAGTAGCCAATGCCCGCCTCGGTTCGTT 60
1 M T I T Y T N K V A N A R L G S F 17

61 CTCGTCCCTCCTCCTGTGCTGGCGAGGCAGCATCTACAAGCTGCTGTATGGAGAATTCCT 120
18 S S L L L C W R G S I Y K L L Y G E F L 37

121 TGTCTTCATATTCCTCTACTATTCCATCCGTGGACTCTACAGAATGGTTCTCTCGAGTGA 180
38 V F I F L Y Y S I R G L Y R M V L S S D 57

181 TCAGCAGCTGTTGTTTGAGAAAGCTGGCTCTGTACTGCGACAGCTACATTCAGCTCATCCC 240
58 Q Q L L F E K L A L Y C D S Y I Q L I P 77

241 TATATCCTTCGTTCTGGGTTTCTATGTTACATTGGTGGTGAGCCGCTGGTGGAGCCAGTA 300
78 I S F V L G F Y V T L V V S R W W S Q Y 97

301 CGAGAACTTGCCGTGGCCCGACCGCCTCATGATCCAGGTGTCTAGCTTCGTGGAGGGCAA 360
98 E N L P W P D R L M I Q V S S F V E G K 117

361 GGATGAGGAAGGCCGTTTGCTGCGGCGCACGCTCATCCGCTACGCCATCCTGGGCAAGT 420
118 D E E G R L L R R T L I R Y A I L G Q V 137

421 GCTCATCCTGCGCAGCATCAGCACCTCGGTCTACAAGCGCTTCCCACTCTTCACCACCT 480
138 L I L R S I S T S V Y K R F P T L H H L 157

481 GGTGCTAGCAGGTTTTATGACCCATGGGGAACATAAGCAGTTGCAGAAGTTGGGCCTACC 540
158 V L A G F M T H G E H K Q L Q K L G L P 177

541 ACACAACACATTCTGGGTGCCCTGGGTGTGGTTTGCCAACTTGCAATGAAGGCCTATCT 600
178 H N T F W V P W V W F A N L S M K A Y L 197

601 TGGAGGTCGAATCCGGGACACCGTCCTGCTCCAGAGCCTGATGAATGAGGTGTGTACTTT 660
198 G G R I R D T V L L Q S L M N E V C T L 217

661 GCGTACTCAGTGTGGACAGCTGTATGCCTACGACTGGATAAGTATCCCATTTGGTGTACAC 720
218 R T Q C G Q L Y A Y D W I S I P L V Y T 237

721 ACAGGTGGTGACAGTGGCAGTATACAGCTTTTTCTTGATGCTTGATCGGGAGGCAGTT 780
238 Q V V T V A V Y S F F L A C L I G R Q F 257

FIG.8A

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781 TCTGAACCCAAACAAGGACTACCCAGGCCATGAGATGGATCTGGTTGTGCCTGTCTTCAC 840
258 L N P N K D Y P G H E M D L V V P V F T 277

841 AATCCTGCAATTCTTATTCTACATGGGCTGGCTGAAGGTGGCAGAACAGCTCATCAACCC 900
278 I L Q F L F Y M G W L K V A E Q L I N P 297

901 CTTGCGGGGAGGACGATGATGATTTTGAGACTAACTGGATCATTGACAGAAACCTGCAGGT 960
298 F G E D D D D F E T N W I I D R N L Q V 317

961 GTCCCTGTTGTCCGTGGATGGGATGCACCAGAACTTGCCTCCCATGGAACGTGACATGTA 1020
318 S L L S V D G M H Q N L P P M E R D M Y 337

1021 CTGGAACGAGGCAGCGCCTCAGCCGCCCTACACAGCTGCTTCTGCCAGGTCTCGCCGGCA 1080
338 W N E A A P Q P P Y T A A S A R S R R H 357

1081 TTCCTTCATGGGCTCCACCTTCAACATCAGCCTAAAGAAAGAAGACTTAGAGCTTTGGTC 1140
358 S F M G S T F N I S L K K E D L E L W S 377

1141 AAAAGAGGAGGCTGACACGGATAAGAAAGAGAGTGGCTATAGCAGCACCATAGGCTGCTT 1200
378 K E E A D T D K K E S G Y S S T I G C F 397

1201 CTTAGGACTGCAACCCAAAACTACCATCTTCCCTTGAAAGACTTAAAGACCAAACCTATT 1260
398 L G L Q P K N Y H L P L K D L K T K L L 417

1261 GTGTTCTAAGAACCCCTCCTCGAAGGCCAGTGTAAGGATGCCAACCAGAAAAACCAGAA 1320
418 C S K N P L L E G Q C K D A N Q K N Q K 437

1321 AGATGTCTGGAATTTAAGGGTCTGGACTTCTTGAAATGTGTTCCAAGGTTTAAGAGGAG 1380
438 D V W K F K G L D F L K C V P R F K R R 457

1381 AGGCTCCCATTGTGGCCCACAGGCACCCAGCAGCCACCCTACTGAGCAGTCAGCACCCCTC 1440
458 G S H C G P Q A P S S H P T E Q S A P S 477

1441 CAGTTCAGACACAGGTGATGGGCCTTCCACAGATTACCAAGAAATCTGTCACATGAAAAA 1500
478 S S D T G D G P S T D Y Q E I C H M K K 497

1501 GAAAACTGTGGAGTTTAACTTGAACATTCCAGAGAGCCCCACAGAACATCTTCAACAGCG 1560
498 K T V E F N L N I P E S P T E H L Q Q R 517

1561 CCGTTTGGACCAGATGTCAACCAATATACAGGCTCTAATGAAGGAGCATGCAGAGTCCTA 1620

FIG.8B

16/18

518 R L D Q M S T N I Q A L M K E H A E S Y 537

1621 TCCCTACAGGGATGAAGCTGGCACCAAACCTGTTCTCTATGAGTGATGCCTCACAGCCTG 1680
 538 P Y R D E A G T K P V L Y E 551

1681 GCCCTGACTTGCAAGGATGCCCAGCAGGGCACTGACCCAGTCAAAGGCACACAAGCAGCG 1740

1741 ACACCCAGGAGTGTGTTCCCACGACAGTCTAGCATGTAACCTCAGAACCAAGAGTACTTAA 1800

1801 TAGTCCTGCCTGAAAACACCTGTATTTTACGATCTTTCCCAAACCTAAGGAGTTT AATAAA 1860

1861 CGTGAATATTCTTTTAGGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1916

FIG.8C

09/622964

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	1		50
Human	MTITYTSQVA	NARLGSFSRL	LLCWRGSIYK LLYGEFLIFL LCYYIIRFIY
MouseBestrophin]	MTITYTNKVA	NARLGSFSSL	LLCWRGSIYK LLYGEFLVFI FLYYSIRGLY
	51		100
Human	RLALTEEQQL	MFEKLTLYCD	SYIQLIPISF VLGFYVTLVV TRWWNQYENL
MouseBestrophin]	RMVLSSDQQL	LFEKLALYCD	SYIQLIPISF VLGFYVTLVV SRWWSQYENL
	101		150
Human	PWPDRLMSLV	SGFVEGKDEQ	GRLLRRTLIR YANLGNVLIL RSVSTAVYKR
MouseBestrophin]	PWPDRLMIQV	SSFVEGKDEE	GRLLRRTLIR YAILGQVLIL RSISTSIVYKR
	151		200
Human	FPSAQHLVQA	GFMTPEAEHQ	LEKLSLPHNM FWVPWVWFAN LSMKAWLGGR
MouseBestrophin]	FPTLHHLVLA	GFMTHEGEHQ	LQKLGLPHNT FWVPWVWFAN LSMKAYLGGR
	201		250
Human	IRDPIQLQSL	LNEMNLTIRT	CGHLYAYDWI SIPLVYTQVV TVAVYSFFLT
MouseBestrophin]	IRDTVLLQSL	MNEVCTLRIT	CGQLYAYDWI SIPLVYTQVV TVAVYSFFLA
	251		300
Human	CLVGRQFLNP	AKAYPGHELD	LVVPVFTFLQ FFFYVGWLKV AEQLINPFGE
MouseBestrophin]	CLIGRQFLNP	NKDYPGHEMD	LVVPVFTILQ FLFYMGWLKV AEQLINPFGE
	301		350
Human	DDDDFETNWI	VDRNLQVSLL	AVDEMHQDLP RMEPDMYWNR PEPQPPYTAA
MouseBestrophin]	DDDDFETNWI	IDRNLQVSLL	SVDGMHQNLP PMERDMYWNE AAPQPPYTAA
	351		400
Human	SAQFRRASF	GSTFNISLNK	EEMEFQPNQEDEEDAH AGIIGRFLGL
MouseBestrophin]	SARSRRHSF	GSTFNISLKK	EDLELWSKEE ADTDKESGY SSTIGCFLGL
	401		450
Human	QSHDHPPRA	NSRTKLLWPK	RESLLHEGLP KNHKAQKQNV RGQEDNKAWK
MouseBestrophin]	QPKNYHLPLK	DLKTKLLCSK	NPLL...EGQC KD.....ANQ KNQKD..VWK
	451		500
Human	LKAVIDAFKSA	PLYQRPGYYS	APQTPLSPTP MFFPLEPSAP SKLHVSITGID
MouseBestrophin]	FRGLDFLKC	PRFKRRGSHC	GPQAPSS... ..HPTEQSAP SS..SDTG..
	501		550
Human	TKDKSLKTVS	SGAKKSFELL	SESDGALMEH PEVSQVRRKT VEFNLDTMPE
MouseBestrophin]DGPSTDY QEICHMKKKT VEFNL.NIPE
	551		596
Human	IPENHLKE.P	LEQSPTNIHT	TLKDHMDPYW ALLENRDEAHS -----
MouseBestrophin]	SPTEHLQQR	LDQMSTNIQA	LMKEHAESY. ..PYRDEAGT KPVLYE

FIG.9

• GCL

IPL

OPL

NO

IS

OS

RPE

FIG.10A

FIG.10B

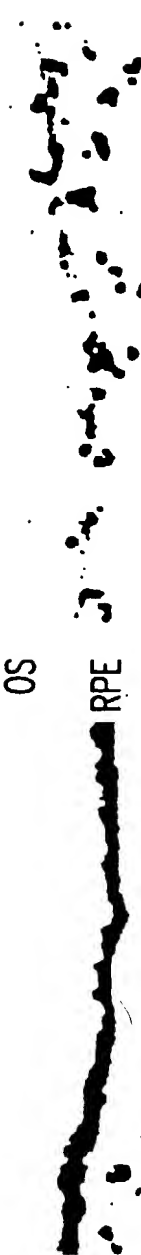
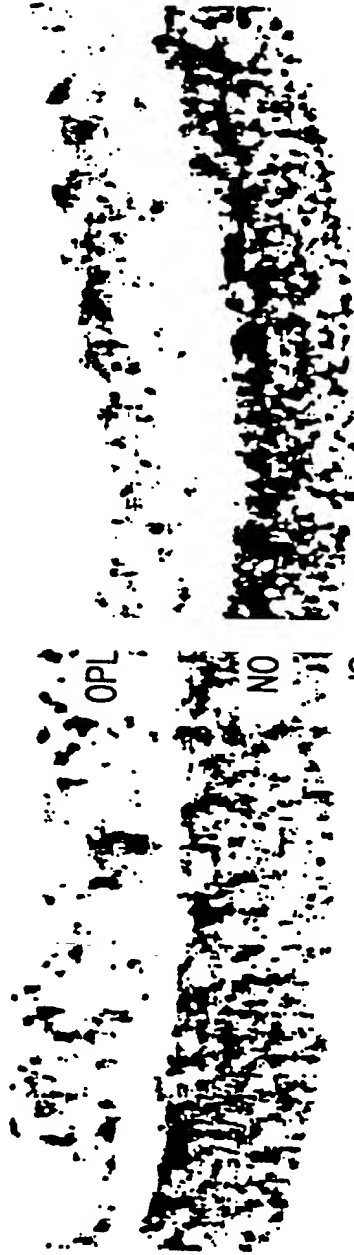


FIG.10C

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PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration
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with Initial
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OR

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(37 CFR 1.16 (e))
required)

Attorney Docket Number

20177YP

First Named Inventor

Petrukhin, et al.

COMPLETE IF KNOWN

Application Number

Filing Date

Group Art Unit

Examiner Name

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BEST'S MACULAR DYSTROPHY GENE

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 08/23/2000 as United States Application Number or PCT International

Application Number 09/622,964 ✓ and was amended on (MM/DD/YYYY) (if applicable).

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Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed? YES NO
				<input type="checkbox"/> <input type="checkbox"/>
				<input type="checkbox"/> <input type="checkbox"/>
				<input type="checkbox"/> <input type="checkbox"/>
				<input type="checkbox"/> <input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/112,926	12/18/1998 ✓	20177PV2
60/075,941	02/25/1998 ✓	20177PV

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U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/US99/03790 ✓	02/22/1999 ✓	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	Joseph A. Coppola				
Address	Merck & Co., Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])				Family Name or Surname			
Konstantin				Petrukhin			
Inventor's Signature	Petrukhin			Date	4 Oct 00		
Residence: City	Collegeville	State	PA	Country	US	Citizenship	RU
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

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SUBSTITUTE for PTO/SB/02A (3-97), Declaration (Additional Inventors)

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
C. Thomas				Caskey			
Inventor's Signature						Date	
Residence: City	Lansdale	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Michael				Metzker			
Inventor's Signature						Date	
Residence: City	Fort Washington	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Claes				Wadelius			
Inventor's Signature						Date	
Residence: City	Upsala	State		Country	Sweden	Citizenship	SE
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

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SUBSTITUTE for PTO/SB/01 (12-97), DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

**DECLARATION AND
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FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	20177YP
First Named Inventor	Petrukhin, et al.
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

BEST'S MACULAR DYSTROPHY GENE

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 08/23/2000 as United States Application Number or PCT International

Application Number 09/622,964 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

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Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

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60/112,926 ✓	12/18/1998 ✓	20177PV2
60/075,941 ✓	02/25/1998 ✓	20177PV

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U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/US99/03790	02/22/1999	

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☐ Customer Number
OR
☒ Registered practitioner(s) name/registration number listed below

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Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

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Name	Joseph A. Coppola				
Address	Merck & Co., Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])				Family Name or Surname			
Konstantin				Petrukhin			
Inventor's Signature					Date		
Residence: City	Collegeville	State	PA	Country	US	Citizenship	RU
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

Please type a plus sign (+) inside this box



DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
C. Thomas				Caskey			
Inventor's Signature				Date	10/30/2000		
Residence: City	Lansdale PA.	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Michael				Metzker			
Inventor's Signature				Date			
Residence: City	Fort Washington	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Claes				Wadelius			
Inventor's Signature				Date			
Residence: City	Uppsala	State		Country	Sweden	Citizenship	SE
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
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Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

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(37 CFR 1.63)**☐ Declaration
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with Initial
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Submitted after Initial
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required)

Attorney Docket Number

20177YP

First Named Inventor

Petrukhin, et al.

COMPLETE IF KNOWN

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Country	USA	Telephone	(732)594-6734	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])				Family Name or Surname			
Konstantin				Petrukhin			
Inventor's Signature				Date			
Residence: City	Collegeville	State	PA	Country	US	Citizenship	RU
Post Office Address	Merck & Co, Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

Please type a plus sign (+) inside this box



Approved for use through 9/30/2000 OMB 651-0032
SUBSTITUTE for PTO/SB/02A (3-97), Declaration (Additional Inventors)

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
C. Thomas				Caskey			
Inventor's Signature						Date	
Residence: City	Lansdale	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
3-02 Michael				Metzker			
Inventor's Signature						Date	10/5/00
Residence: City	Fort Washington	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Claes				Wadelius			
Inventor's Signature						Date	
Residence: City	Upsala	State		Country	Sweden	Citizenship	SE
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
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Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		

Please type a plus sign (+) inside this box

+

SUBSTITUTE for PTO/SB/01 (12-97), DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

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**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration
Submitted
with Initial
Filing

OR

☒ Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number

20177YP

First Named Inventor

Petrukhin, et al.

COMPLETE IF KNOWN

Application Number

Filing Date

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

BEST'S MACULAR DYSTROPHY GENE

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 08/23/2000 as United States Application Number or PCT International

Application Number 09/622,964 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
YES	NO				
<input type="checkbox"/>	<input type="checkbox"/>				
<input type="checkbox"/>	<input type="checkbox"/>				
<input type="checkbox"/>	<input type="checkbox"/>				
<input type="checkbox"/>	<input type="checkbox"/>				

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/112,926	12/18/1998	20177PV2
60/075,941	02/25/1998	20177PV

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/US99/03790 ✓	02/22/1999 ✓	

☐ Additional U S or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number OR
☒ Registered practitioner(s) name/registration number listed below

Place Customer Number
Bar Code Label here

Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	<u>Joseph A. Coppola</u>				
Address	<u>Merck & Co., Inc. - Patent Department</u>				
Address	<u>P.O. Box 2000, RY60-30</u>				
City	<u>Rahway</u>	State	<u>NJ</u>	ZIP	<u>07065-0907</u>
Country	<u>USA</u>	Telephone	<u>(732)594-6734</u>	Fax	<u>(732)594-4720</u>

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Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname			
Konstantin		Petrukhin			
Inventor's Signature				Date	
Residence: City	Collegeville	State	PA	Country	US
				Citizenship	RU
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	State	NJ	ZIP	07065-0907

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Please type a plus sign (+) inside this box



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DECLARATION AND POWER OF ATTORNEY

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Inventor's Signature						Date	
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City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Michael				Metzker			
Inventor's Signature						Date	
Residence: City	Fort Washington	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
H-01 Claes				Wadelius			
Inventor's Signature						Date	October 16, 2000
Residence: City	Uppsala	State	SE	Country	Sweden	Citizenship	SE
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
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Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		